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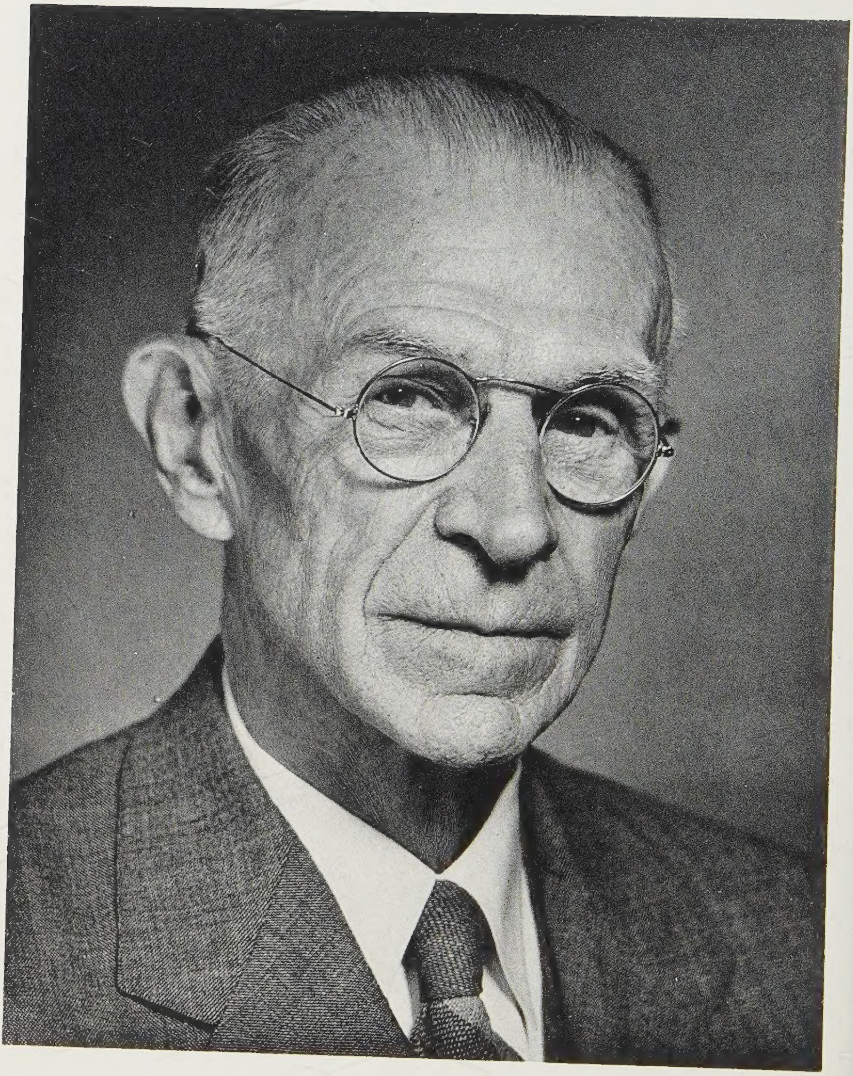


IRVING WIDMER BAILEY

DEDICATION

On the occasion of the retirement of PROFESSOR IRVING WIDMER BAILEY *after twenty-two years on the staff of the Arnold Arboretum and forty-seven years of service to Harvard University, we, his associates on the editorial board of the Journal of the Arnold Arboretum, dedicate this issue to him.*

The authors of the articles in this special number of the Journal represent only a few of the students and associates of Professor Bailey who would pay tribute to him at this time. We honor him with this issue in recognition of his contributions, his high standards of research, his integrity in science and personal life, and his guidance of and inspiration to students in the field of botany.

JOURNAL

OF THE

ARNOLD ARBORETUM

VOL. XXXVI APRIL–JULY 1955 NUMBERS 2 & 3

THE COMPARATIVE MORPHOLOGY AND RELATIONSHIPS OF THE MAGNOLIACEAE — IV. WOOD AND NODAL ANATOMY

JAMES E. CANRIGHT

With three plates

INTRODUCTION

THE MORPHOLOGICAL AND ANATOMICAL CHARACTERISTICS of the magnolia family have attracted the interest of many investigators over a long period of time. This interest stemmed not only from the supposed antiquity of the Magnoliaceae, but also from the group's phylogenetic importance in many systems of classification.

Some of the earliest work on the anatomy of the Magnoliaceae was that of Matsuda (34), Gröppler (25), and Parmentier (42). Undoubtedly the most detailed description of any magnoliaceous wood was by Moll and Janssonius (37) on seven Javanese species. Solereder's (51) anatomical descriptions were based almost entirely on twig material removed from herbarium sheets, therefore the recent publication of Metcalfe and Chalk (36) is of considerably more value, since mature woods of five of the ten genera of Magnoliaceae (*sensu stricto*) were examined. McLaughlin (35) has made one of the most important contributions, due to the fact that his wood descriptions were based on a study of 40 species (of some 200 now recognized for this family). Other papers bearing on this topic by Lemesle (31) and Ozenda (41) have recently appeared.

Despite all of the above-mentioned reports, the true phylogenetic value of the woods of the Magnoliaceae, in the light of modern knowledge, still remains to be fully evaluated.

In 1940, following 30 years of intensive anatomical research on such basic problems as the ontogenetic and phylogenetic significance of the cambium and its derivatives, nodal anatomy, cell wall structure, and many related subjects, Professor Irving W. Bailey launched a series of cooperative investigations on both reproductive and vegetative organs of dicot families belonging to the order Ranales (*sensu* Engler). These extensive surveys were made possible by Prof. Bailey's long and active interest in building up the Harvard Slide Collections; these included not only a large

and diversified assemblage of wood, pollen, and leaves, but also some floral organs and seedlings. In this connection, it goes without saying that the living collections and herbarium specimens of the Arnold Arboretum were an invaluable source of much of the plant material.

As a result of the Bailey-stimulated interest in the Ranales, during the past 13 years a number of comprehensive papers by Bailey, his colleagues and students have appeared. These papers delineate the morphology, trends of specialization, and natural relationships of the following woody ranalean families: Degeneriaceae (8, 54); Winteraceae (4); Himantadraceae (7); Trochodendraceae and Tetracentraceae (5, 49); Eupteleaceae (39); Illiciaceae and Schisandraceae (6, 50); Cercidiphyllaceae (55); Magnoliaceae (11, 12); Austrobaileyaceae (9); and Monimiaceae (38).

Indeed, an examination of recent taxonomic works, e.g., Gunderson (26), Stebbins (52), pp. 8–9, and Lawrence (29), clearly indicates the profound influence the findings of the "Bailey school" have had on present-day concepts of the woody Ranales.

The present paper is respectfully dedicated to Prof. I. W. Bailey upon the occasion of his retirement from active teaching, since it was he who first fostered my interest in ranalean plants, and who has encouraged and helped me in innumerable ways.

MATERIALS AND METHODS

As interpreted by Dandy (17), the Magnoliaceae include 10 genera and perhaps as many as 210 valid species. *Magnolia* is the largest genus, comprised of about 80 temperate and tropical species, of which 24 are American and the rest Asiatic in distribution. *Talauma,* containing about 42 species, also is characterized by a split distribution; however, only nine species occur in tropical America, with the remainder being scattered throughout Malaysia. *Liriodendron* has two temperate species — one in eastern North America and one in central China. All other genera normally grow within an area extending from the Himalayas to China and Japan, southward through Burma, Malaya, Indo-China, Siam, Indonesia, and the Philippines, to northern New Guinea. *Michelia* includes about 50 species; *Manglietia,* 25; *Elmerrillia,* 7; *Aromadendron,* 3; *Kmeria,* 2; *Pachylarnax,* 2; and *Alcimandra* is monotypic.

In the survey of mature woods of this family, transverse, radial, and tangential sections were examined from 61 species in seven genera. In addition, transverse and longitudinal sections were made from twigs (4–6 mm. in diameter) from herbarium specimens of the rare genera *Alcimandra* and *Kmeria.* Thus, only *Pachylarnax* was not represented. This investigation included all slides and wood samples of the Magnoliaceae in the Harvard wood collection, as well as the collections of the Yale School of Forestry.[1]

In order to check the ranges of variability of the wood characters in each genus, 5″ × 8″ punch cards (Y–9 Unisort Analysis Cards from the Chas.

[1] The writer is indebted to the curators of these collections for the loan of material.

R. Hadley Co., Los Angeles) were utilized for each species. Around the margins of these cards are 94 numbered perforations which were keyed to the most important diagnostic features of woods as listed by Record and Chattaway (45). In examining each wood slide microscopically, whenever a certain feature was present, the numbered hole on the card referring to this feature was notched. After the entire wood survey was completed, this notch allows the card to drop out of the stack whenever a spindle is inserted in that hole, thereby indicating the presence of that particular feature in that species. The punch card method also proved of value in the revelation of several wood specimens which had apparently been wrongly identified in the past. In the event that herbarium vouchers are not in existence for questionably-identified wood slides, this punch card method offers one of the best means of identification.

The number of wood macerations made was insufficient to allow reliable utilization of vessel element length as a feature of phylogenetic importance. The wood ray types proposed by Kribs (27) and the wood parenchyma classification of Bailey and Howard (3) are followed here; in all other cases the terminology follows that recommended by the Committee on Nomenclature of the International Association of Wood Anatomists (15).

Transverse nodal sections of 25 species were cut serially, commencing from below the node and passing up through the petiole, in order to ascertain the vascular pattern. The majority of these twigs were sectioned unembedded at 24μ on a sliding microtome. However, due to their extreme hardness, twigs of about ten species (mainly talaumas and manglietias) had to be softened in hydrofluoric acid and embedded in celloidin before sectioning was possible.

The serial order of the nodal and petiolar sections was maintained by placing the sections in a linear sequence on a glycerine-covered slide immediately after each section was cut. The slide was then covered by a piece of fine mesh stainless steel wire cloth, which was held on by means of a wooden clip. This wire cloth did not appreciably interfere with later dehydration or staining procedures. The sections were then stained in safranin or else mounted unstained in diaphane.

ANATOMICAL DESCRIPTIONS OF THE GENERA

Of the 40 species examined by McLaughlin (35), 35 belonged to the genera *Magnolia*, *Michelia*, and *Talauma*. Since McLaughlin's descriptions of these three genera are substantially correct, the present treatment will refer to these genera only whenever our observations seem to be at variance, or else to supplement portions of the previous descriptions.

Alcimandra Dandy

Diffuse porous; growth rings indistinct; pores angular in cross section, thin-walled, small (ca. 47μ in diameter), solitary to radial chains of 2–3, numerous, *Fig. 5*; perforation plate scalariform, 8–15-barred; intervascular pitting scalariform with traces of opposite pitting in older wood; wood

parenchyma apotracheal, in tangential bands 2–4 cells wide; fiber tracheids, pits with crossing slit-like apertures whose length equals the diameter of the pit border; wood rays, Heterogenous Type I — tall uniseriates composed mainly of upright cells, and tall (up to 100 cells) and narrow (2–3 cells) multiseriates; oil cells absent. Description based on *Alcimandra cathcartii* (Hk. f. & Thoms.) Dandy collected by *J. D. Hooker* without number from Sikkim (H–27724).

Alcimandra cathcartii (Hk. f. & Thoms.) Dandy was originally referred to the genus *Michelia* on account of the stipitate gynoecium and character of the fruit, but Dandy (17) separated it out because it was the only *Michelia* species characterized by terminal flowers. The wood of this plant is essentially like that of *Michelia* with the following exceptions: the pores are smaller, there are more bars in the perforation plate; the rays are taller, and ethereal oil cells are lacking. However, these differences, for the most part, may be ascribed to the lack of maturity of the *Alcimandra* material examined, i.e., the differences may be ontogenetic not phylogenetic ones.

Aromadendron Blume

Diffuse porous; growth rings faintly visible (due to slight thickening of radial walls in late wood elements); pores essentially solitary, oval in outline, very large (up to 240μ in tangential diameter), *Fig. 1*; perforation plate scalariform, 6–10-barred; intervascular pitting scalariform, with some reticulations, *Fig. 22*; tyloses not uncommon; spiral thickenings present; wood parenchyma terminal or initial in rows of 4–5 cells; fiber tracheids, with crossed apertures slightly extended; Heterogeneous Type IIA rays; multiseriates 2–3 cells wide and 15–40 cells high, with oil cells of fairly common occurrence on their margins; oil cells may also infrequently occur within body of uniseriate rays composed of upright cells, *Fig. 16*.

Above description based on mature wood of *A. elegans* Bl. (H–22787) and twig material (H–27725).

McLaughlin's (35) statement that the wood of this genus contains no marked characteristics separating it from that of *Talauma* is not substantiated by the present study. The pores in *Aromadendron* are the largest in the entire family, and are almost twice the size of the pores in any of the investigated Asiatic *Talauma* spp. Differences are also apparent in the wood parenchyma distribution and fiber tracheid characteristics of the two genera. Finally, a cursory comparison of floral and fruit types is sufficient in this case to refute McLaughlin's unigeneric views.

Elmerrillia Dandy

Diffuse porous; growth rings usually indistinct; pores solitary, *Fig. 2* (but with some tendency towards formation of radial chains), oval in outline, medium to large (135–220μ); perforation plate scalariform, 4–10-barred; intervascular pitting scalariform; parenchyma terminal or initial, in rows of 3–4 cells; fiber tracheids with pit apertures included or slightly

extended; rays, Heterogeneous IIA and IIB (complete elimination of uniseriates in *E. mollis*); multiseriate rays mainly biseriate and 15–40 cells high; oil cells common in margins of rays, *Figs. 13, 15.*

Description based on *E. mollis* Dandy (H–25694), *E. ovalis* (Miq.) Dandy (H–25695 and Y–19509), and *E. papuana* (Schltr.) Dandy (H–27728).

The wood of this genus falls within the range of variability of the woods of *Michelia*, which *Elmerrillia* also most closely resembles in exomorphic characters.

Kmeria Dandy

Diffuse porous; pores solitary to multiples, numerous, *Fig. 6*; vessel diameter small (52μ); perforation plate scalariform, 8–12-barred; intervascular pitting scalariform; parenchyma thick-walled, apparently terminal, in rows of 2–3 cells; fiber tracheids; rays heterogeneous (exact size not determinable); oil cells absent.

Above description based on 1½ yr. old twig of *K. duperreana* (Pierre) Dandy, 6 mm. in diameter (H–27730) from Indochina.

Although the wood of this genus is indistinguishable from that of many tropical *Magnolia* spp., Dandy's (17) transfer of *Magnolia duperreana* into a new genus based on its possession of unisexual flowers seems valid in this otherwise hermaphroditic family.

Liriodendron L.

The wood of this genus is sufficiently well known, therefore only a brief comparison of the anatomy of the American and Chinese species seems warranted. In general the two woods are quite similar, but the following differences are noted: in *L. chinense* the pores tend to be less numerous, more rounded, thicker-walled, and smaller in diameter (85μ) than those of *L. tulipifera* (120μ in diameter). The difference in thickness of walls of the fiber tracheids and the diameter of the pores in the late wood as compared to the early wood is much more pronounced in *L. chinense* than in the American species. The size of the early wood pores of the Chinese species is almost twice that of the late wood pores, whereas in the American species there is no appreciable difference in early and late wood pore size, *Fig. 7.* There are more and larger pits (border = 7μ) in the walls of the fiber tracheids of *L. chinense*, whereas the fiber tracheid pits in *L. tulipifera* average only 3.5μ and the apertures are commonly included. Tyloses are numerous in vessels of *L. tulipifera* and absent in *L. chinense*. The 2–3-seriate rays of the latter species tend to be fairly uniform in size, rarely ranging over 20 cells high. On the other hand, the rays of *L. tulipifera* tend to be slightly broader (3–4-seriate) and often reach heights of 25–35 cells, mixed in with many shorter rays, *Fig. 17.*

Material examined: *L. chinense* (Hemsl.) Sargent (Y–21461, H–27731): *L. tulipifera* L. (H–3759, H–24492, H–24675, and H–24674).

It is entirely possible that insufficient sampling makes these differences

in the wood of the two species more apparent than real. Nevertheless, the information should at least be taken into consideration by certain systematists who feel that the exomorphic characters of the Chinese species are insufficient bases for specific rank.

Magnolia L.

Some outstanding features of the wood of this familiar genus are: growth rings clearly apparent in the temperate species due to the prominent bands of terminal (or initial) parenchyma, but inconspicuous in many tropical species, e.g., *M. paenetalauma* from Hainan, *Fig. 4*. This species is also noteworthy in that it possesses the smallest pores (48μ) seen in mature woods of this family. The pore diameter of all other investigated species in this genus ranges from 65–120μ. With the exception of *M. poasana* (Pitt.) Dandy, which is characterized by solitary, sparsely-distributed pores (only 8–10/ sq. mm.), the pores of *Magnolia* spp. are numerous, crowded, and often in dense radial chains or clustered. Although the majority of magnoliaceous species have scalariform perforation plates (6–12-barred), simple perforations in oblique end walls occur in certain temperate species — *M. acuminata, M. campbelli* (*Fig. 10*), *M. cordata* (*Fig. 9*), *M. kobus* DC., *M. obovata*, *M. soulangeana* (*M. denudata* Desr. × *M. liliflora* Desr.), and *M. stellata* Max. Tyloses rare, but present in *M. cordata*, *M. pterocarpa*, and *M. tripetala*. Spiral thickenings prominent only in *M. grandiflora*, *M. hamori*, and *M. paenetalauma*. Intervascular pitting scalariform to transitional, with only *M. fraseri* having strictly opposite pitting. Parenchyma apparently terminal (3–8 rows) in temperate species, but the narrow-banded apotracheal type is not uncommon in some tropical species, e.g. *M. cubensis*; in most species the radial walls of the parenchyma strands are highly sculptured, *Fig. 18*. Pits on fiber tracheids may have either included or extended slit-like apertures. Rays Heterogeneous Type IIA and IIB, except in three temperate species which are characterized by more advanced Homogeneous Type II rays (*M. acuminata*, *M. kobus*, and *M. obovata*). Oil cells seen only in rays of *M. cubensis*, *M. hamori*, and *M. splendens* — all from the West Indies.

Wood examined from 22 species as follows: *M. acuminata* L. (H–17352, H–18963); *M. campbelli* Hk. f. & Thoms. (H–4395L); *M. cordata* Sarg. (H–8968); *M. cubensis* Urb. (Y–19283); *M. fraseri* Walt. (H–17353); *M. grandiflora* L. (H–8972, H–17355); *M. gustavii* King (H–27770); *M. hamori* Howard (*Hamor* without number); *M. javanica* Koord. & Val. (H–17356); *M. kobus* DC. (H–17359); *M. macrophylla* Michx. (H–8979); *M. obovata* Thunb. (H–8981, H–8974); *M. paenetalauma* Dandy (H–21111); *M. parviflora* Sieb. & Zucc. (H–27736); *M. poasana* (Pitt.) Dandy (Y–38423); *M. pterocarpa* Roxb. (H–17360); *M. soulangeana* Hort. (H–21018); *M. splendens* Urb. (H–1713); *M. stellata* Max. (H–21306); *M. tripetala* L. (H–8983, H–17362); *M. virginiana* L. (H–1712, H–8970).

Manglietia Blume

Growth rings indistinct; pores solitary to radial chains of 2–5; vessel diameter in mature wood generally 120–155μ; perforation plate scalariform, 5–12-barred; intervascular pitting scalariform to transitional, *Fig. 23*; parenchyma terminal or initial in rows 2–6 cells wide; fiber tracheids, pit apertures included or slightly extended; rays of Heterogeneous Type IIA, mainly 2–3-seriate and up to 40 cells high; oil cells absent.

A wood supposedly belonging to *M. fordiana*, *Fig. 24*, (det. by W. C. Cheng of the Fan Inst. of Biology) differs from the above generic description in several important characters; pores small (60μ), numerous, crowded together in no definite pattern; scalariform perforation plate only 1–6-barred; growth rings distinct; Heterogeneous IIB rays.

Material examined: *M. caveana* Hk. f. & Thoms. (H–27743); *M. chevalieri* Dandy (H–27744); *M. conifera* Dandy (H–27746); *M. dandyi* Gagnep. (H–27747); *M. fordiana* Oliv. (H–27748, Y–31907); *M. glauca* Bl. (Y–30038); *M. hookeri* Cub. & W. Sm. (H–27750); *M. insignis* (Wall.) Bl. (Y–9580, H–27752).

McLaughlin's statement (35) that *Manglietia* may be only a subgenus of *Talauma* gains no support from this investigation. Indeed, if any fusion of genera were attempted, it would seem more logical to combine *Manglietia* with *Magnolia*. There is little difference in the wood anatomy of *Manglietia* and many temperate *Magnolia* spp.; in addition, the only clear-cut difference in floral characters lies in the number of ovules/carpel — (2 in *Magnolia*, and 4–14 in *Manglietia*).

Michelia L.

Minute anatomy as described in McLaughlin (35) with the following exceptions or addenda: pores solitary or in radial chains of 2–5, except *M. maudiae* and *M. lanuginosa* which have pores in clusters; pore diameter of largest elements in mature woods ranges from 70μ in *M. maudiae* to 220μ in *M. lanuginosa*; perforation plates always scalariform, 1–6-barred, *Fig. 8*; tyloses occur only in *M. baillonii*, *M. balansae*, and *M. oblonga*; spiral thickenings very common in 8 of 12 examined species (absent only in *M. figo*, *M. lanuginosa*, *M. montana*, and *M. oblonga*); distribution of wood parenchyma is more variable in this genus than in any other in this family; although terminal or initial bands (in 2–6 rows) are most common, *M. figo* has closely-spaced single rows of apotracheal parenchyma, and *M. champaca* has some scanty paratracheal, ranging to abaxial types in the late wood of *M. baillonii*, *Fig. 21*; fiber tracheids usually thick-walled and with pit apertures more or less extended; rays Heterogeneous Type IIA and IIB; uniseriates rare (to none in *M. tsoi* and *M. baillonii*), never over 10 cells high; multiseriates 2–3 cells wide, rarely over 25 cells high, and usually bearing oil cells among the upright marginal cells.

Material examined: *M. baillonii* (Pierre) F. & G. (Y–13251); *M. balansae* (A. DC.) Dandy (Y–12914); *M. champaca* L. (H–1016); *M.*

compressa (Max.) Sarg. (H–23170); *M. foveolata* (Merr.) Dandy (H–21081); *M. fuscata* Bl. (H–15777); *M. figo* (Lour.) Spreng (Y–22012); *M. lanuginosa* Wall. (Y–30043); *M. maudiae* Dunn (H–21085); *M. montana* Bl. (Y–19506, H–17365); *M. oblonga* Wall. (Y–9570); *M. tsoi* Dandy (H–21079).

The wood from China which was identified as *M. figo* (Y–22012, Fan Memorial Inst. #0544) has a number of anatomical features, including alternate intervascular pitting, which are inconsistent with other woods in this family, let alone the genus *Michelia*. Although *M. fuscata* Bl. is putatively a synonym for *M. figo* (Lour.) Spreng., due to the many anomalous features found in the wood specimen supposedly belonging to the latter species, the present author was unable to verify the synonymy. Unfortunately, no herbarium voucher was deposited with this Chinese wood specimen; thus a recheck on the original determination was impossible in this case.

Talauma Juss.

Growth rings absent; pores solitary to some radial chains of 2–3, rounded or oval; pore diameter varies from 65μ (*T. hodgsoni*) to 205μ (*T. gloriensis*); perforation plate scalariform, 8–20-barred; intervascular pitting scalariform with some transitional; tyloses present in 8 of the 12 examined species, and some sclerotic tyloses were found in *T. angatensis*, *T. gigantifolia*, *T. sambuensis*, and *T. villariana*; spiral thickenings present only in *T. mexicana*; parenchyma apotracheal narrow-banded (1–4 cells), bands often closely-spaced, being only 170μ apart in *T. villariana*, *Fig. 25*; fiber tracheids, thick-walled, pit borders much reduced and apertures greatly extended; rays of Heterogeneous Type I and IIA, uniseriate rays composed of upright cells, multiseriates up to 4 cells wide and 40 high; oil cells occur in rays of seven species.

Above description based on examination of: *T. angatensis* (Blanco) F. Vill. (Y–19995, H–27761); *T. candollei* Bl. (H–15783); *T. dodecapetala* (Lam.) Urb. (H–27763); *T. gigantifolia* Miq. (H–3761J); *T. gloriensis* Pitt. (Y–38448); *T. hodgsoni* Hk. f. & Thoms. (H–15787); *T. mexicana* Don. (H–27765); *T. minor* Urb. (Y–19313); *T. mutabilis* Bl. (H–17377); *T. rumphii* Bl. (Y–19510); *T. sambuensis* Pitt. (H–1711, from type tree); *T. villariana* Rolfe (Y–17148).

DISCUSSION OF WOOD ANATOMY

Just as in the case of the stamens (11) and pollen (12) of this family, there is a considerable degree of generic overlap in the characters of phylogenetic importance. [For a concise summation of the evolutionary trends known for woods, see Tippo (56)]. In other words, all the primitive characters are not confined to any one genus, nor are all the advanced characters. Nevertheless, on the basis of the present wood survey, there are many indications that the woods of the temperate species of *Magnolia* and *Lirio-*

dendron are the most specialized, with most tropical species of *Talauma* exhibiting the largest assemblage of primitive characters. As previously noted in the generic descriptions of the woods, certain temperate species of *Magnolia* have the following advanced [2] characters: numerous crowded pores, porous perforation plates, Homogeneous Type II rays, and in one case, strictly opposite intervascular pitting. *Liriodendron* also shows this last-named feature. On the other hand, certain species of *Talauma* are characterized by the following primitive features: solitary sparsely-distributed pores, up to 20-barred perforation plates, elongate vessel elements, apotracheal narrow-banded parenchyma, and Heterogeneous I or IIA rays.

It is of interest to note a rough correlation between the stage of evolution represented by the woods of many temperate magnolias with that determined independently by Dandy (18) based solely on exomorphic characters. The most advanced *Magnolia* subgenus (Pleurochasma) contains three sections (*Yulania*, *Buergeria*, and *Tulipastrum*) which include such anatomically-advanced species as *M. acuminata*, *M. cordata*, *M. kobus*, *M. campbelli*, and *M. stellata*. By way of comparison, *Magnolia paenetalauma* from tropical Hainan exhibits a number of primitive features, such as small solitary pores, long vessel elements with many-barred (up to 16) perforation plates, and extremely tall (over 100 cells) heterogeneous rays. Dandy (*loc. cit.*) has placed this species in the first section (*Gwillimia*) of the more primitive subgenus *Magnolia*; in so doing he remarked that the members of this section resemble those of the genus *Talauma* in so many ways that the generic position of some of the species is uncertain in the absence of fruiting material.

However, it should be emphasized that this apparent correlation between external and internal characters is not always present, since rates of floral and vegetative evolution do not necessarily progress at the same pace. In this connection, Bailey (2) has recently warned of the dangers inherent in jumping to conclusions concerning plant phylogeny and classification based on inadequate anatomical data from a single organ or tissue. Unfortunately, in working on the woods of the Magnoliales, McLaughlin (35) fell into this pitfall; although his observations are basically sound, he assumed too much from the examination of wood specimens alone. For example, he recommended that *Aromadendron* and *Manglietia* be reduced to subgenera of *Talauma*, and that *Elmerrillia* and *Alcimandra* be similarly reduced to *Michelia*. These proposed major shifts in the classification of this family were based solely on the evidence gleaned from not more than five wood specimens from the four above-mentioned "doubtful" genera. Furthermore, McLaughlin reduced 10 species to synonymy without benefit of study of herbarium specimens. In view of situations of this type, it is little wonder that certain taxonomists — specialists on one group or another — have little respect for anatomical research; in fact, may even literally lock their doors (and herbarium cases) whenever an anatomist

[2] Applied here in the sense that these characters are advanced in relation to the wood characters in the family as a whole. No magnoliaceous woods are highly evolved when compared to those of all angiosperm families.

approaches and requests their cooperation and advice. As Bailey (1) has pointed out, due to the widely-occurring cases of parallel evolution in the woods of dicotyledons, evidence from the vascular tissues, by itself, proves to be more significant in negations than in affirmative conclusions.

Lemesle (30, 31) claimed discovery of a new type of tracheary element in the xylem of the ranalean genera *Schisandra*, *Kadsura*, and *Illicium*. This type of element was distinguished from the more common fiber tracheids in other ranalean genera by the fact that these "cycad-like tracheids" were characterized by bordered pits whose crossed apertures were usually smaller than the borders. He further postulated that this type of element was much more archaic than the fiber tracheids of the Magnoliaceae, since the latter elements had circular bordered pits whose crossed apertures equaled or exceeded their borders. Finally, upon the unsubstantiated assumption that the characters of the fibers are more constant and unchangeable than those of the vessels, Lemesle (30) proposed a linear phylogenetic sequence, deriving the Magnoliaceae from the Bennettitales by way of the "Proangiosperms", "Homoxyleae" [Van Tieghem's (57) unnatural grouping of vesselless angiosperms], Schisandraceae, and Illiciaceae.

Although the present author has recorded for the fiber tracheids of the Magnoliaceae whether the pit apertures are generally included or extended, no phylogenetic import is attached to these features. Indeed, it seems probable that the size of the obliquely oriented slit-like apertures depends to a certain extent on the amount of shrinkage of the wood samples caused by drying prior to sectioning. The oblique or helical orientation of the microfibrils of cellulose in the secondary walls of tracheary elements, as observed by Bailey and Vestal (10) and Maby (32), lends considerable support to this conclusion.

The distribution of the wood paranchyma in the Magnoliaceae is commonly referred to as terminal (36, 46). This type, according to Kribs (28), represents a specialization due to reduction, since there is no correlation with any one vessel type. Furthermore, this type of parenchyma distribution occurs indiscriminately throughout many dicotyledonous families.

Coster (16) mentions his belief that terminal parenchyma formation has a direct physiological relationship to the annual drop of leaves in deciduous forms. Obviously, however, this explanation cannot be valid for non-deciduous tropical members of the Magnoliaceae characterized by terminal parenchyma. Chowdhury (13, 14) has made ontogenetic investigations of diffuse porous tropical woods putatively bearing terminal parenchyma; he has been able to show in many cases (e.g., *Terminalia tomentosa*, *Dahlbergia sissoo*, *Swietenia macrophylla*, *Salix matsudana*, etc.) that the wood parenchyma bands actually form in the *beginning* of each year's growth ring, thus should be referred to as initial, not terminal. In the light of this evidence, the present author preferred to use the phraseology "terminal or initial" in describing the wood parenchyma of most magnoliaceous species, at least until such time as this feature can be thoroughly investigated from an ontogenetic standpoint. The fact remains, however, that both terminal

and initial patterns of parenchyma distribution are undoubtedly specialized types, conceivably having been formed by means of a reduction series from either paratracheal or apotracheal types. Therefore, the usual absence of terminal or initial parenchyma in the wood of examined species of *Talauma* indicates another primitive character for the wood of this genus.

The phylogenetic value of spiral thickenings on the secondary wall of vessels is still a controversial matter. Frost (22) and Stern (53) regard spiral thickenings as an advanced character, yet Metcalfe and Chalk (36) from an examination of over 3000 woods state that this feature is about twice as common in unspecialized as in specialized woods. Further, the latter authors' statistics reveal that spiral thickening is often linked with ring porosity, occurring in 44% of the ring porous woods, and only in 6½% of diffuse porous woods. Record (44) states that spiral thickenings are much less common in tropical woods than in temperate woods, which concurs with the above statistics of Metcalfe and Chalk. Petersen (43), after a comparison of woods belonging to the advanced Amentiferae and the primitive Ranales, found this feature equally divided between the two groups.

The data from the woods of the Magnoliaceae lend little support to either concept regarding the significance of spiral thickenings. This feature was present in vessels of only 14 species of 61 examined, and was more common in *Michelia* (in eight of 12 species) than in any other genus. Although wood of *Michelia* seems to be more specialized (viz., 1–6-barred perforation plates, occurrence of pore multiples, thick-walled fiber tracheids, and tendency toward paratracheal parenchyma) than other tropical magnoliaceous genera, the sampling is insufficient to warrant an interpretation of the phylogenetic value of spiral thickenings. In addition, the fact that Metcalfe and Chalk (*loc. cit.*) list this character for 101 widely separated families of dicotyledons, tends to further invalidate conclusions of evolutionary importance.

Similarly, the presence or absence of tyloses is only of diagnostic, and not phylogenetic, value. This feature was found in 18 species, being most common in *Talauma* (in eight of 12 examined species), where four species even exhibited sclerotic tyloses.

Oil cells are common features in the rays of most magnoliaceous species, being absent only in examined species of *Alcimandra*, *Kmeria*, *Liriodendron*, and *Manglietia*. However, since oil cells are also of common occurrence throughout all organs of most ranalean families, their presence in woods of the Magnoliaceae is of no special significance.

As previously noted by Bailey et al (7), the woods of the Degeneriaceae, Himantandraceae, and Magnoliaceae are of a basically similar type, differing markedly from that which occurs in other ranalean families, with the possible exception of the Annonaceae. The wood of Degeneriaceae is of a comparatively primitive type; that of the Magnoliaceae shows transitional stages from the *Degeneria* type up to comparatively advanced types with porous vessels and homogeneous rays; the wood of *Himantandra* most

closely compares with some of the more advanced members of the Magnoliaceae in its possession of both scalariform and porous vessels, plus intervascular pitting transitional between opposite and alternate. The regular occurrence of sclerotic pith diaphragms in all three families is also noteworthy.

PHLOEM

Esau et al (21) have recently discussed the manifold problems involved in attempting to establish reliable trends of specialization for phloem tissues. One of these problems concerns the collapse of leptom (soft-walled) tissue upon drying, which naturally makes observation of its details difficult, if not impossible.

When living secondary phloem is properly fixed, the alternate stratification of leptom and fibrous tissue is plainly evident in certain temperate magnoliaceous species, e.g., *Liriodendron tulipifera, Fig. 3.* Furthermore, the prominent sieve tube elements and companion cells are separated from the thick-walled phloem fibers by tangentially aligned uni- or biseriate rows of phloem parenchyma. A similar arrangement has been observed in phloem of *L. chinense*, *Manglietia* spp., and *Michelia champaca.* However, this precise arrangement of phloem tissues is less clear in other tropical species of the Magnoliaceae (perhaps due in part to poor preservation of phloem in the examined species), and seems to be absent entirely in *Alcimandra.* In the latter genus bands of phloem fibers extend radially, instead of tangentially, more or less flanking the sieve tube elements. Therefore, although the evidence is admittedly fragmentary, it is suggested that the occurrence of alternate tangential banding of leptom and fibrous tissues in the secondary phloem of dicotyledons may be a further trend of specialization.

Among the Magnoliaceae the conspicuous V-shaped flaring of the phloem rays (as viewed in cross section) is usually associated with the type of tangentially banded phloem mentioned above. It is significant that such V-shaped rays and alternate hard and soft bast layers have already been noted in the Degeneriaceae (8) and Himantandraceae (7).

MacDaniels (33) has described the sieve tube element of *Magnolia acuminata* and *Liriodendron tulipifera* as being characterized by 6–10 rounded sieve areas situated on an oblique end wall. This type of sieve tube element he termed Type II, i.e., slightly more specialized than Type I (very oblique end walls containing 10–20 sieve areas). Thus, for these two species the level of specialization for this phloem element roughly parallels that of the vessel elements. However, in view of the fact that the woods of these two species are among the most advanced in the family, it would seem desirable to withhold final judgment on this matter until well-preserved phloem of some of the more primitive tropical magnoliaceous species can be thoroughly investigated.

NODAL ANATOMY

The nodal anatomy of the Magnoliaceae is rendered quite complex due to the constant occurrence of sheathing stipules together with a large number of stipular traces. However, in the node of members of the tribe Magnolieae (of Dandy, 17) there is usually only one trace whose sole function is the vascularization of the stipular sheath;[3] this trace is located on the opposite side of the cauline stele from the large median trace to the leaf, *Fig. 19*. In the case of *Liriodendron tulipifera* (tribe Liriodendreae) Ozenda (40) has demonstrated that each of the six lateral traces also sends small amounts of vascular tissue into the stipular sheath. Nevertheless, since the main vascular supply of these lateral traces passes almost horizontally around the stele and into the margins of the petiole, when serial sections are examined the median and lateral traces are readily distinguishable from the stipular trace.

In the base of the petiole of all examined species the traces split up in a variable fashion, and some of the bundles may become inverted. No matter how variable the pattern in the base of the petiole, the bundles ultimately form a ring as they near the base of the lamina. The occurrence of a few medullary and/or cortical bundles in the petiole of some species is not uncommon.

Table 1 indicates the results of a survey of the vegetative node of 25 species in 8 genera of the Magnoliaceae. These figures illustrate the extreme variability in the number of nodal bundles, not only between different species in a genus, but also between different nodes from the same specimen.

These observations do not agree, for the most part, with those of Ozenda (41), who found the nodal pattern in this family relatively stable for most genera. For purposes of comparison, Ozenda's (*loc. cit.*, p. 128) list of leaf trace numbers is as follows: *Michelia*, 3 or 5; *Liriodendron*, *Alcimandra*, and *Pachylarnax*, 7; *Talauma*, *Magnolia*, and *Manglietia*, 7 or 9; *Kmeria* and *Elmerrillia*, 9. (Apparently these numbers are exclusive of the stipular trace). The difference between these and our own observations would seem to be caused by the fact that the number of leaf traces in the Magnoliaceae varies considerably with the stage of ontogeny. For example, the first and second seedling leaves in the Magnoliaceae are vascularized by only three traces, i.e., the nodes at this stage are trilacunar. Older leaves add more traces, with the number gradually becoming more stabilized on the mature parts of the tree.

A similar variation in nodal condition was reported by Swamy (54) for *Degeneria*. Here, although the nodes of the mature stem are pentalacunar, the first few leaves of the seedling are vascularizd by only three traces. In addition, large leaves from vigorously growing saplings may receive more than five traces from the corresponding node. Furthermore, numerous cases could be cited for other families which have mature trilacunar nodes but whose seedling nodes are unilacunar.

[3] Ozenda (41) reports two stipular traces for *Magnolia grandiflora* L.

TABLE 1

Species Examined	Number of Traces: Median	Lateral	Stipular	Totals for Genus
Alcimandra cathcartii (Hk. f. & Thoms.) Dandy	1	8	1	10
Elmerrillia mollis Dandy	1	12	1	14–17
E. ovalis (Miq.) Dandy	1	15 *	1	
Kmeria duperreana (Pierre) Dandy	1	7–8	1	9–10
Liriodendron chinense (Hemsl.) Sarg.	1	6	1	8
L. tulipifera L.	1	6	1	
Magnolia acuminata L.	1	10	1	5–12
M. coco (Lour.) DC.	1	4	1	
M. javanica Koord. & Val.	1	6	1	
M. nitida W. W. Sm.	1	6	1	
M. salicifolia Maxim.	1	3–5	1	
M. virginiana L.	1	6	1	
Manglietia blaoensis Dandy	1	8	1	8–10
M. conifera Dandy	1	6	1	
M. hainanensis Dandy	1	6	1	
M. insignis (Wall.) Bl.	1	7	1	
M. thamnodes Dandy	1	6	1	
Michelia baillonii (Pierre) F. & G.	1	5	1	6–10
M. compressa (Maxim.) Sarg.	1	4	1	
M. doltsopa (Buch.-Ham.) DC.	1	5	1	
M. montana Blume	1	6–8	1	
Talauma hodgsoni Hk. f. & Thoms.	1	8	1	6–10
T. mutabilis Blume	1	4–5	1	
T. ovata St. Hil.	1	6	1	
T. sambuensis Pittier	1	8	1	

* Whenever an odd number of laterals is listed, it indicates that one bundle of a pair has been suppressed; close checks on lower sections failed to reveal its presence in these cases.

This evidence, as well as that recorded by Money et al (38) for the node of the Monimiaceae, makes it increasingly important that we reexamine Sinnott's (47) concepts concerning the phylogenetic significance of nodal anatomy. The main facts and conclusions of Sinnott (from Sinnott and Bailey, 48) are summarized as follows: 1. Three fundamental types of nodal anatomy occur — unilacunar, trilacunar, and multilacunar, depending upon the number of gaps left in the cauline stele by the departure of bundles to the leaf; 2. These three types are exceedingly constant within

the major taxonomic groups, i.e., orders and families; 3. The trilacunar type (*Text-figure 1–C*) is regarded as the most primitive, not only due to its predominance in the lower Archichlamydeae, but also because of its occurrence in the least specialized members of otherwise multi- or unilacunar families or orders; 4. The uni- and multilacunar nodes are derived from the trilacunar type respectively through a reduction series on one hand, and an amplification of lateral bundles on the other; and 5. The multilacunar node of monocotyledons has apparently been derived from such a trilacunar condition as persists in the Potamogetonaceae.

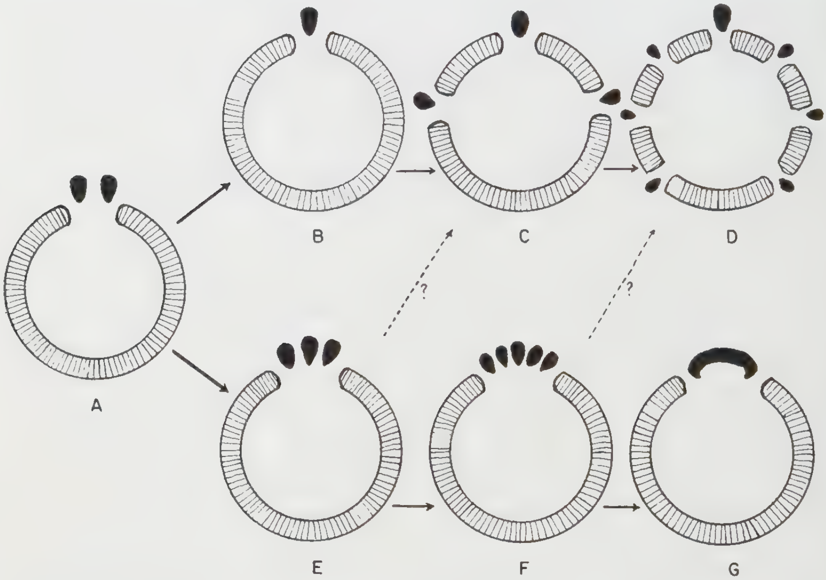
Sinnott's premises Nos. 1 and 2 are chiefly factual and have therefore become well-established and universally accepted concepts over the course of the past 40 years. On the other hand, premises Nos. 3, 4, and 5 are largely based on interpretations of the taxonomic distribution of the nodal types; it is these interpretations which are now being questioned. In other words, which of the three nodal patterns is the primitive type, and which are the derived?

Ozenda (41, p. 136) has taken the position that the three nodal types in angiosperms should be interpreted as a strict reduction series, i.e., the multilacunar type is primitive, the trilacunar or pentalacunar node is intermediate, and the unilacunar node is the most advanced. (In *Text-figure 1* this interpretation would be DCB.) Furthermore, in order to explain the occurrence of multilacunar nodes in such highly evolved groups as the Umbelliflorae, Asterales, Polygonales, etc., Ozenda has adopted the theory of *surévolution* of Gaussen (23, 24). Briefly, the basic tenets of this theory are that whenever an avowedly primitive feature is found in a plant (or animal) which is otherwise highly specialized, this apparently primitive feature is merely analagous, not homologous, to the truly primitive one, having previously passed through a complete evolutionary cycle.

However, if we are to accept the interpretation that the multilacunar node is primitive for dicotyledons, we are also forced to accept the corollary that this condition must have been inherited from either the Cycadaceae or the Monocotyledonae, the only other groups characterized by multilacunar nodes. Despite the superficial resemblance of the girdling leaf traces of the cycads to a similar condition for the lateral traces in the Magnoliaceae, *Fig. 20*, we are forced to reject any suggestion of relationship between these two groups due to a wide divergence of reproductive structures, as well as major differences in the xylem. Similarly, the fossil evidence plus the complexity of both reproductive and vegetative organs of the monocotyledons make it illogical to derive the multilacunar nodes of dicotyledons from monocotyledons. It might be mentioned in passing that Worsdell (58) attempted to derive dicotyledons from monocotyledonous plants, e.g., palms, in the belief that the "grandifoliate" habit of the latter group was a primitive feature. Little importance is attached to such a theory today.

If we reject the premise that the multilacunar node is primitive and the unilacunar node is advanced, what evidence exists which might lead us to assume that the reverse may be true?

First of all, among the lycopsids, ferns, seed ferns, cordaites, ginkgos, and conifers the leaf traces are either single or double (though splitting into more in some ferns). In certain members of ancient pteropsids, e.g., *Lyginopteris*, *Mesoxylon*, *Ginkgo*, there are normally two leaf traces confronting a single gap for each leaf. No leaf gap exists in the lycopsids, but a single gap occurs for the bulk of the non-angiosperm tracheophytes with the following exceptions: according to Eames (20) the node in *Ephedra* is bilacunar, although some species reportedly have two traces arising side by side; in *Welwitschia* two traces originally arise from one gap but accessory traces are added lateral to and between the original bundles as the persistent leaves widen through the years; the condition in *Gnetum* is reportedly multilacunar, 4 traces from 4 gaps (Duthie, 19) or 7–11 traces from an equal number of gaps (Sinnott, 47). Therefore, excluding



TEXT-FIGURE 1. Suggested phylogenetic trends of nodal types in angiosperms: A, two traces from a single gap represents the primitive type; B, a common unilacunar type resulting from the fusion of the two traces in the most primitive type; C, the trilacunar type resulting from an addition of two lateral traces and accompanying leaf gaps; D, a multilacunar type probably derived from the previous type by the addition of more laterals (plus a stipular trace in the case of the Magnoliaceae); E, a unilacunar type with three traces, derived from most primitive type by the splitting of one trace; F, same as E except for an increased number of traces from the single gap; G, a unilacunar type with a single broad trace derived from type F through fusion of 5–7 traces from a single gap. Thus, a unilacunar node may represent a fairly low level of specialization in the cases of types A and B, or a highly-evolved condition in the case of type G. The trilacunar and multilacunar types may, in some cases, have arisen from the unilacunar types E and F.

the highly aberrant "Chlamydosperms," it is evident that the fundamental nodal type in groups below the angiosperms on the evolutionary scale is one with a single leaf gap confronted by one or two leaf traces.

Among dicotyledons Bailey (38, p. 384) has indicated that the most common pattern of cotyledonary vascularization is one where each cotyledon is supplied by two independent traces that are related to a single gap. A strikingly similar type of vascularization has been reported by Bailey and Swamy (9) for the mature nodes of *Austrobaileya*. It is emphasized that these two leaf traces do not originate as a dichotomy of a single bundle, but instead are attached to two independent parts of the stele below the nodal level. Money et al (38) have found the same nodal condition in *Trimenia* and *Piptocalyx* (now separated out as the Trimeniaceae); the nodes of the more advanced members of the related Monimiaceae are characterized by either 3, 5, or 7 traces from a single gap, or in the case of the Siparuneae, a single broad arc-shaped trace.

Sinnott (47) has shown an ontogenetic sequence for *Chenopodium album* where the young node has three traces from a single gap, but through fusion forms a broad single trace in the mature node. Members of the Ebenaceae, Myrtaceae, and Ericaceae also show a single broad trace at the nodal level.

In summarizing the foregoing paragraphs, the following points are emphasized: 1. Nodal types in the vascular plants below the evolutionary level of the angiosperms are prevailingly unilacunar; in many cases two traces may confront a single gap; 2. In known ontogenetic sequences for certain dicotyledons (Magnoliaceae, Degeneriaceae), there is an increase in the number of leaf traces, and this tendency is usually correlated with a corresponding increase in the number of leaf gaps; 3. The basic pattern of vascularization for the majority of dicots at the level of the cotyledonary node is one with two traces from a single gap; these two traces are *not* the result of a bifurcation of a single trace further down in the hypocotyl; 4. An apparently phylogenetic sequence seen in the mature unilacunar node of the Monimiaceae (*sensu lato*) involves a series commencing with two traces (*Austrobaileya*, *Trimenia*, *Piptocalyx*), followed by types with 3, 5, or 7 traces (*Hortonia*, *Anthobembix*, *Mollinedia*), and ending with the leaf trace represented by a single broad arc (Siparuneae).

Therefore, it is suggested (See *Text-Fig. 1*) that the primitive nodal type for angiosperms is unilacunar with two traces, although these traces may sometimes be fused. By addition of laterals the trilacunar and multilacunar types evolve; these are regarded as increasing specializations of the basic unilacunar pattern. In cases of unilacunar nodes with a single broad arc of vascular tissue representing the leaf trace, this nodal type is believed to have become secondarily specialized as the end result of ontogenetic or phylogenetic fusion of several smaller traces from a single gap.

It is acknowledged that a thorough reinvestigation of the vascular pattern of both the cotyledonary and mature nodes of many widely-separated angiosperm families will be necessary before the many facets of this hypothesis can be adequately tested, and the results reliably

utilized in studies of phylogeny. Nevertheless, the present author firmly believes that sufficient evidence is now available to warrant the conclusion that the multilacunar node, as exemplified by that of the Magnoliaceae, is a highly specialized feature.[4]

Indeed, supplementary evidence of trends of evolutionary specialization from many other parts of the plant — wood, phloem, seedling anatomy, and carpels (ovules often reduced to two, and cohesion and adnation of common occurrence) — clearly indicate that the Magnoliaceae can no longer be regarded as the most primitive of angiosperms. Furthermore, the tissues and organs of the Magnoliaceae reveal many transitions between the more primitive Degeneriaceae and the slightly more specialized Himantandraceae; yet all three families undoubtedly form a compact alliance within the woody Ranales.

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EXPLANATION OF PLATES

PLATE I

FIGURES 1–7. FIG. 1. *Aromadendron elegans* Bl. (H–22787), transverse section of wood showing very large vessels, × 50. FIG. 2. *Elmerrillia mollis* Dandy (H–25694), transverse section of wood illustrating large thin-walled vessels, a band of parenchyma, and secretory cells, × 70. FIG. 3. *Liriodendron tulipifera* L. (H–24675), transverse section of secondary phloem showing tangential banding of sieve tube elements and phloem fibers. (Cambial zone near bottom of figure). × 75. FIG. 4. *Magnolia paenetalauma* Dandy (H–21111), cross-section of wood showing small angular vessels, apotracheal narrow-banded parenchyma, and broad primary rays. × 75. FIG. 5. *Alcimandra cathcartii* (Hk. f. & Thoms.) Dandy (H–27724), portion of twig cross-section, × 75. FIG. 6. *Kmeria duperreana* (Pierre) Dandy (H–27730), transverse section showing portion of first two growth rings, × 75. FIG. 7. *Liriodendron tulipifera* L. (H–3759), transverse section of wood showing terminal parenchyma and similarity of late and early wood elements, × 40.

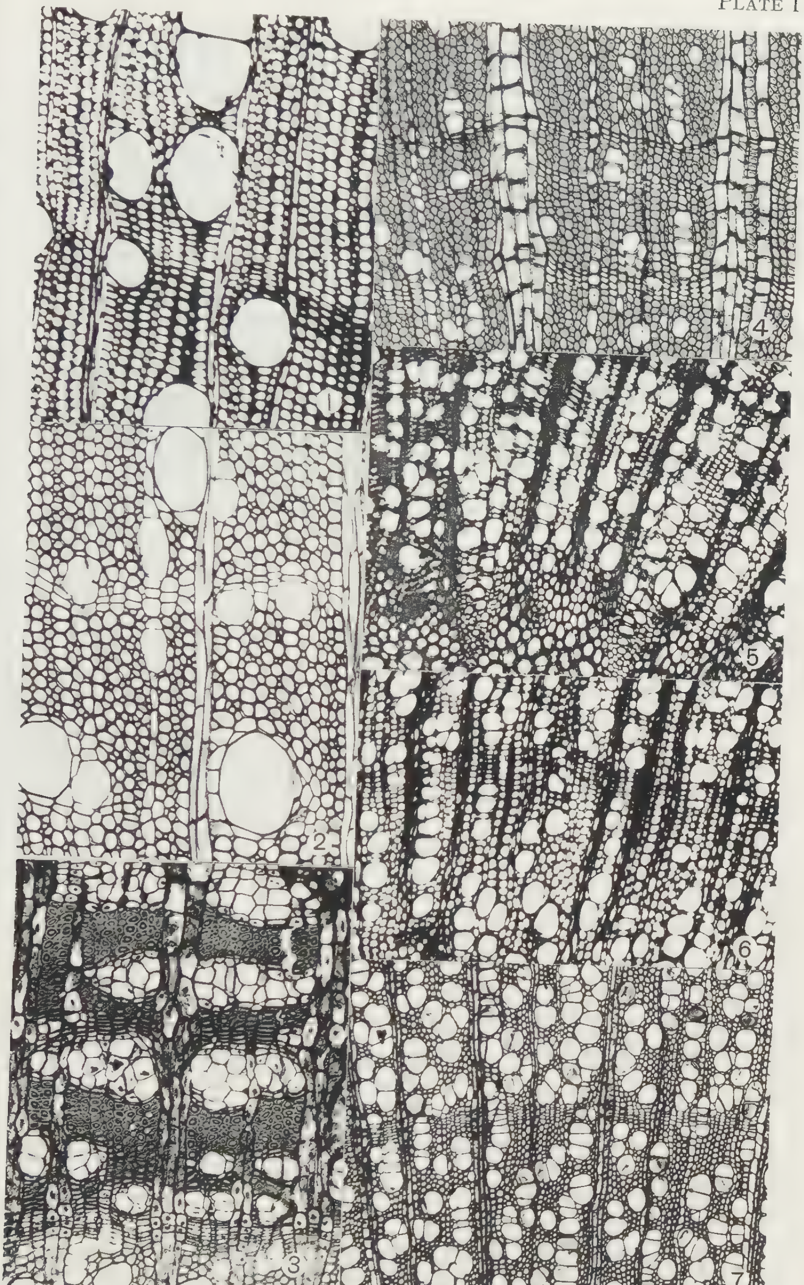
PLATE II

FIGURES 8–19. FIG. 8. *Michelia fuscata* Bl. (H–15777), radial section showing few-barred perforation plates and spiral thickenings, × 100. FIG. 9. *Magnolia cordata* Sarg. (H–8968), radial view of end of vessel element showing porous perforation resulting from loss of bars, × 160. FIG. 10. *Magnolia campbelli* Hk. f. & Thoms. (H–4395L), large obliquely-oriented pore in end wall of vessel element and transitional intervascular pitting, × 150. FIG. 11. *Magnolia acuminata* L. (H–17352), tangential section showing transitional intervascular pitting, fiber tracheids, and biseriate rays, × 65. FIG. 12. *The same*, anomalous septations in fiber tracheids, × 70. FIG. 13. *Elmerrillia ovalis* (Miq.) Dandy (Y–20574), radial view showing oil cells both in margins of rays and in strand parenchyma, × 35. FIG. 14. *Magnolia paenetalauma* Dandy (H–21111), tangential section showing large number of uniseriate rays and narrow tracheid-like vessel elements, × 75. FIG. 15. *Elmerrillia ovalis* (Miq.) Dandy (Y–20574), tangential section showing fairly low rays and sparse vessel distribution, × 35. FIG. 16. *Aromadendron elegans* Bl. (H–22787), radial view showing scalariform vessel-ray pitting and oil cell in body of uniseriate ray of upright cells, × 65. FIG. 17. *Liriodendron tulipifera* L. (H–3759), tangential view showing tyloses in vessels and nature of rays, × 40. FIG. 18. *Magnolia virginiana* L. (H–8970), tangential view showing heavily-sculptured walls of wood parenchyma cells, × 110. FIG. 19. *Liriodendron chinense* (Hemsl.) Sarg. (H–27731), nodal section

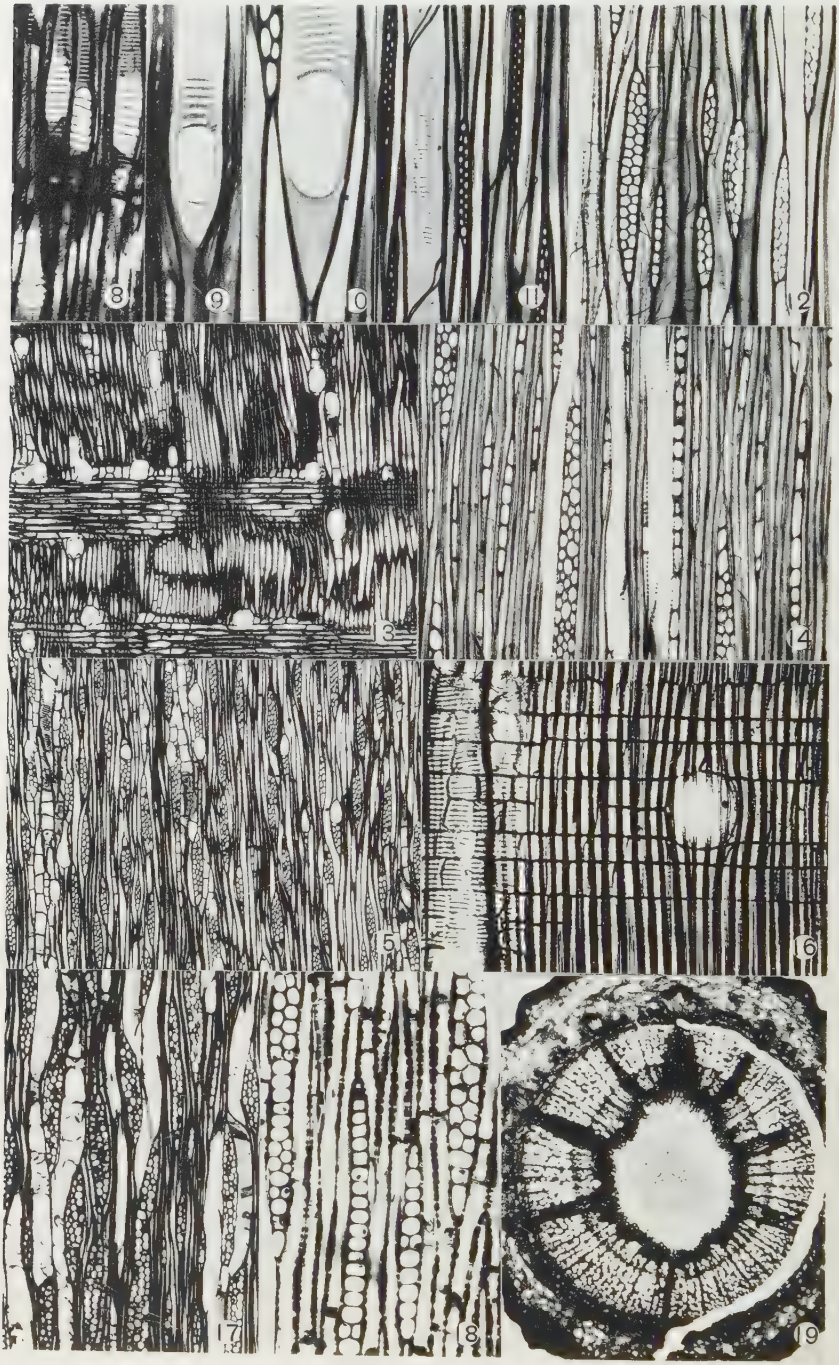
showing large median trace, three pairs of laterals, and one small stipular trace, × 12.

PLATE III

Figures 20–25. Fig. 20. *Magnolia fraseri* Walt., cleared young branch showing mode of vascularization of three spirally-arranged leaves. Dark flecks are sclereid clusters in cortex, × 8. Fig. 21. *Michelia baillonii* (Pierre) F. & G. (Y–13251), transverse section of wood showing wide band of apotracheal parenchyma and some paratracheal parenchyma, × 70. Fig. 22. *Aromadendron elegans* Bl. (H–22787), tangential view showing nature of intervascular pitting, × 85. Fig. 23. *Manglietia glauca* Bl. (Y–30038), tangential view showing transitional intervascular pitting and narrow rays, × 70. Fig. 24. *Manglietia fordiana* Oliv. (Y–31907), transverse section showing crowded angular vessels, thin-walled fiber tracheids, and two bands of terminal (?) parenchyma, × 70. Fig. 25. *Talauma villariana* Rolfe (Y–17148), transverse section showing vessel distribution and numerous bands of apotracheal parenchyma, × 70.



Canright, Anatomy of Magnoliaceae



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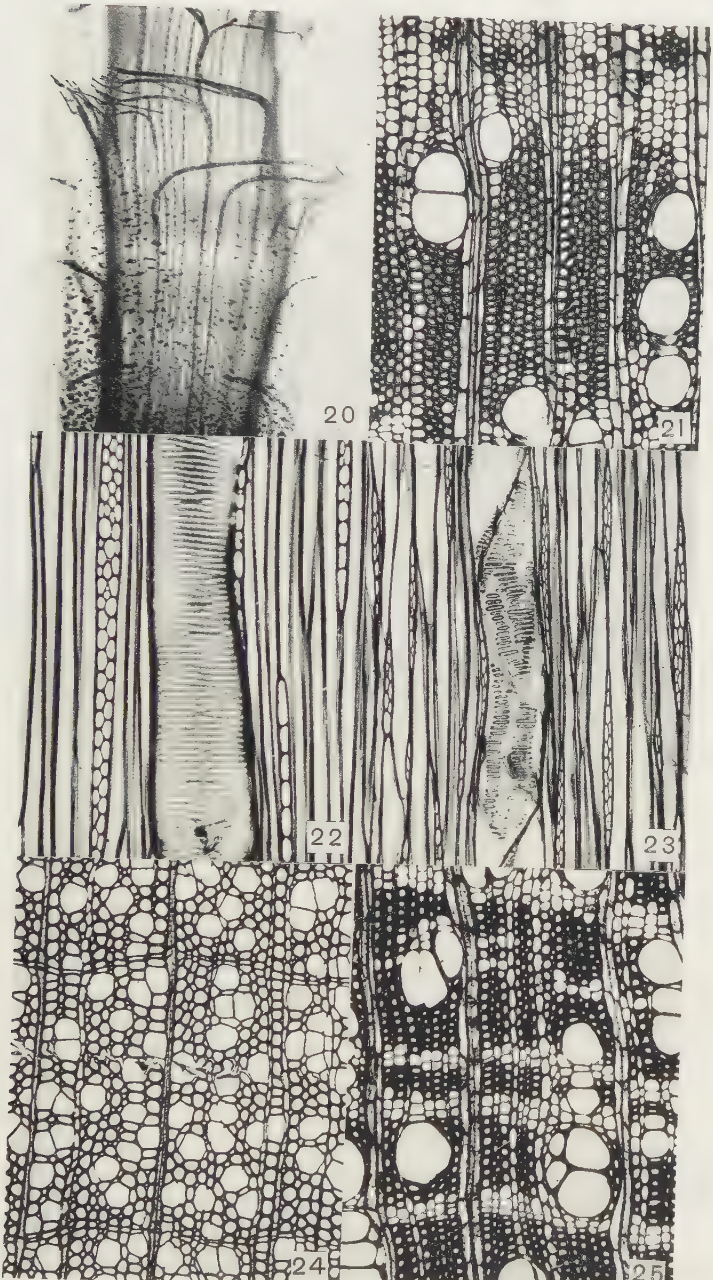


Canright, Anatomy of Magnoliaceae

THE TAXONOMIC USE OF SPECIALIZATION OF VESSELS IN THE METAXYLEM OF GRAMINEAE, CYPERACEAE, JUNCACEAE, AND RESTIONACEAE

Vernon I. Cheadle

With one plate

INTRODUCTION

Enough proof of the usefulness of the vessel as a tool in phylogenetic and taxonomic studies has been given in the literature (e.g. Bailey, 1944; Cheadle, 1944; Tippo, 1946) to justify use of this measuring device in such investigations. Important studies of this nature in the dicotyledons (e.g. Bailey and Howard, 1941; Heimsch, 1942; Tippo, 1938) have been published, but with considerable emphasis on other taxonomic characters as well. If information concerning vessels in many families must always await publication only in conjunction with other available taxonomic facts, however, it is likely that such information will be extremely slow in appearing. Data of a reliable nature on secondary xylem of the stems in woody dicotyledons — such as appear, for example, in the journal *Tropical Woods* — are available for many families and there are published compendia that relate these data and other information to classification (e.g. Metcalfe and Chalk, 1950). For herbaceous dicotyledons and for monocotyledons especially, on the other hand, published information on vessels in taxa, even on the family level, is indeed scanty.

In the writer's opinion, therefore, the information at hand for families in the Monocotyledoneae should be made available for those taxonomists who may find it useful in helping to solve certain thorny questions of genetic affinity. Some examples of the possibilities of thus using the data in this report will be suggested in the Discussion and Conclusions. It seems especially pertinent to supply such information because it is now reasonably clear that vessels have arisen independently in the Monocotyledoneae (Cheadle, 1953). The vessel in the Monocotyledoneae, properly used, thus can be an independent tool — used without regard to what has happened in specialization of vessels outside the monocotyledons — in phylogenetic studies within this group of plants. If the independent origin of vessels is accepted, for example, there is no longer any advantage, each time the vessel situation in a group of monocotyledons is elucidated, in examining groups within the dicotyledons for comparable levels of specialization of vessels with the hope of suggesting, on this basis, some common relationship between the monocotyledons and dicotyledons. The modern concepts of the phylogenetic origin and specialization of vessels in the Monocotyledoneae, furthermore, were developed outside the influence of any preconceived notions (a) regarding what plants on other grounds are

considered primitive in the monocotyledons or (b) from what source the monocotyledons arose. Information on vessels in the Monocotyledoneae accordingly may be of considerable value to taxonomists, especially those interested in the monocotyledons as a whole, for use in manuals (e.g. Hutchinson, 1934) or in textbooks (e.g. Lawrence, 1951) in which phylogenetic relationships are considered.

This paper will be chiefly a report on vessels in such species as have become available up to the present in the four families Gramineae, Cyperaceae, Juncaceae, and Restionaceae. Certain of the author's information, either heretofore unpublished or not used in the same way, about the Liliaceae is also included to provide more obvious examples of the use of specialization of vessels for taxonomic purposes. Information from the Liliaceae was chosen because (a), as will be shown later, tracheary elements in this family on the average are so much less specialized than those in the four families chiefly discussed and (b) primitive members of the Liliaceae are often considered as part of the basic stock from which a number of groups of families arose (Hutchinson, 1934, p. 6). These derivatives include the four families discussed in this paper. An especially wide range of representatives of such a critical family as the Liliaceae should be investigated before tracheary elements in the family can be reliably described. Use of data from the Liliaceae in this paper, however, is such that further investigations of the family would not change the conclusions drawn. It is regrettable that for many of the species there are no herbarium vouchers, but any uncertainty concerning taxa relates only to species within genera; where there has been the slightest doubt about generic identifications,* after consultations with taxonomists, the results have been excluded. Sampling within the Gramineae and Cyperaceae is fairly representative; for the other two families many genera remain to be examined before reliable generalizations can be made. Data have been accumulated, however, for all available organs, species by species, and any one who wishes the detailed information may obtain it by corresponding with the writer.

Data from certain other families will be reported when a few more genera are examined. In the meantime, it would be helpful if plant anatomists would describe accurately the vessels in the plants — in either the monocotyledons or dicotyledons — they use for experimental or descriptive purposes, even if only a single species is involved. If the vessel turns out to be as reliable a measure of specializations as it now appears, this structure certainly should be accurately described in as many species as possible, and particularly in "critical" species where vessels could possibly serve to invalidate, or help to confirm, questionable taxonomic groupings.

MATERIALS AND METHODS

The number of species in which either roots, stems, inflorescence axes, or leaves of the plant were examined in each of the four families is shown

* Except for *Pleioblastus*.

in TABLE 1. The families are named as described by Hutchinson (1934). Where possible, Hitchcock (1951) was followed for generic names in the Gramineae and Fernald (1950) for genera in the Cyperaceae and Juncaceae. Hutchinson (1934) was followed for all other generic titles. Data from 53 species in 40 genera of the Liliaceae as understood by Hutchinson (1934) were used in establishing the place of the Liliaceae in text-figures 1–3.

The number of species studied in each genus occurs within the parentheses following each genus in the following tabulation. Totals are: Gramineae, 50 genera, 69 species; Cyperaceae, 13 genera, 36 species; Juncaceae, 2 genera, 10 species; Restionaceae, 6 genera, 12 species.

Gramineae

Agropyron (1)	*Deschampsia* (1)	*Phleum* (1)
Agrostis (1)	*Digitaria* (1)	*Phragmites* (1)
Ammophila (1)	*Echinochloa* (2)	*Phyllostachys* (5)
Andropogon (3)	*Glyceria* (4)	*Pleioblastus* (1)
Anthoxanthum (1)	*Gynerium* (1)	*Pleuropogon* (1)
Arundinaria (1)	*Hilaria* (1)	*Poa* (2)
Arundo (1)	*Holcus* (1)	*Secale* (1)
Avena (1)	*Hordeum* (1)	*Setaria* (1)
Bouteloua (1)	*Leersia* (1)	*Sorghum* (1)
Bromus (1)	*Lolium* (1)	*Spartina* (3)
Calamagrostis (1)	*Melica* (1)	*Sporobolus* (3)
Cenchrus (1)	*Muhlenbergia* (1)	*Thysanolaena* (1)
Chloris (1)	*Neyraudia* (1)	*Tripsacum* (1)
Chusquea (2)	*Oryzopsis* (1)	*Triticum* (2)
Dactylis (1)	*Panicum* (3)	*Zea* (1)
Danthonia (1)	*Paspalum* (1)	*Zizania* (1)
Dendrocalamus (1)	*Phalaris* (1)	

Cyperaceae

Bulbostylis (1)	*Dulichium* (1)	*Rhynchospora* (3)
Carex (9)	*Eleocharis* (4)	*Schoenus* (1)
Caustis (1)	*Eriophorum* (2)	*Scirpus* (4)
Cladium (2)	*Mesomelaena* (1)	*Scleria* (1)
Cyperus (6)		

Juncaceae

Juncus (8)	*Luzula* (2)

Restionaceae

Anarthria (1)	*Leptocarpus* (1)	*Lyginia* (2)
Hypolaena (2)	*Loxocarya* (4)	*Restio* (2)

Macerations were made of practically all materials using techniques described by Cheadle (1942). About three-quarters of the organs have also been sectioned. Permanent slides were prepared in all instances.

Except for the Restionaceae, most of the species were collected in and around Rhode Island. A considerable number of species is included from several other geographical areas, however, including New York, New Mexico, California, Florida, Alabama, Panama, and Australia. It is a pleasure to express thanks to all those who have supplied material. Professor A. J. Eames deserves special mention for collecting in Australia all the species studied in the Restionaceae.

OBSERVATIONS

The terms used in the following section are chiefly used as defined and illustrated in Esau (1953). For the purposes of this paper, protoxylem is considered as that area in which the tracheary elements have either annular or helical thickenings or both, metaxylem as all the remaining primary xylem. Early metaxylem is that which matures first after protoxylem and late metaxylem that which matures last (Cheadle, 1944, p. 83). Data from the shoot system were taken from the larger bundles. Small bundles need further investigation. Figures 1, 2 and 3 in Plate I illustrate the categories of metaxylem in root and shoot. Plate I, figure 7 provides examples of simple perforation plates, and figures 4–6 and 8–10 various types of scalariform plates.

The level of specialization of vessels is measured by the character of their perforation plates. The validity of this procedure has been discussed by Cheadle (1943b, p. 485). As was pointed out by the same author (1943a), specialization of the perforation plate proceeds from long scalariform plates with many bars (and perforations) on oblique end walls to simple plates (with single perforations) on transverse end walls of vessel members. Thus there is a gradation from many perforations through fewer and fewer until a single large perforation results. Scalariform perforation plates with some of their bars incompletely formed are examples of stages in this phylogenetic change; such plates frequently occur in the Cyperaceae, Juncaceae, and Restionaceae. In spite of this gradation, for ease in presentation all the degrees of specialization within the scalariform type itself have been considered as one category (scalariform). Hence the differences shown in table 1 and text-figures 1–3 are minimum differences.

Although text-figures 1–3 show the data in an easily understood fashion, they do not provide any evidence for variation in vessel specialization in each organ considered in the four families. This variation is expressed in the percentages placed in table 1 and will be discussed first.

A. Variation in Occurrence of Types of Perforation Plates in the Several Organs

The data in table 1 demonstrate variations in the kinds of perforation plates found in the late metaxylem of the several organs of the plant in the four families under consideration. The pattern of variation in the early

metaxylem is generally comparable to that in the late metaxylem, except that it occurs at a lower average level of specialization (cf. Cheadle, 1944). While there are several important features in the table that might be discussed, the only one which needs special attention — because it is not revealed in text figures 1–3 — can be adequately treated as an answer to the following question. Are there species in each of the various families whose perforation plates are strikingly different from the average conditions in the family, and if so, in what organs do these differences occur?

If attention is turned first to the Gramineae, it is obvious that in roots, scalariform plates seldom occur and then only in company with far more

TABLE 1

Percentages of Species in Which Vessel Members with Various Types of Perforation Plates Occur in the Late Metaxylem of Roots, Stems, Inflorescence Axes, and Leaves

	No. spp.	Perforation Plates				
		Simple only	Mostly simple	Simple and scalariform	Mostly scalariform	Scalariform only
Roots						
Gramineae	56	96.4	3.6	0.0	0.0	0.0
Cyperaceae	32	46.9	50.0	3.1	0.0	0.0
Juncaceae	9	55.5	22.2	11.1	11.1	0.0
Restionaceae	10	20.0	40.0	30.0	10.0	0.0
Stems						
Gramineae	69	79.7	20.3	0.0	0.0	0.0
Cyperaceae	34	8.8	82.4	2.9	0.0	5.9
Juncaceae	9	0.0	40.0	30.0	30.0	0.0
Restionaceae	12	8.3	33.4	50.0	0.0	8.3
Inflorescence Axes						
Gramineae	42	61.9	38.1	0.0	0.0	0.0
Cyperaceae	16	6.2	75.0	0.0	12.5	6.2
Juncaceae	8	0.0	25.0	37.5	37.5	0.0
Restionaceae	11	0.0	18.2	72.7	0.0	9.1
Leaves						
Gramineae	60	55.0	43.3	1.7	0.0	0.0
Cyperaceae	29	0.0	82.8	0.0	3.4	13.8
Juncaceae	7	0.0	14.3	14.3	57.1	14.3
Restionaceae	11	0.0	9.1	45.4	18.2	27.3

numerous simple plates. In the remainder of the plant, scalariform plates become progressively more common until in the leaves of one species (1.7 per cent) about equal numbers of simple and scalariform plates occur. As a matter of fact, under the Mostly simple column the number of scalariform plates is almost always very small; in the organs of some species only 2 or 3 were observed and these had few bars. Species in the

Gramineae obviously are so specialized in the late metaxylem that no great variation occurs.

If species in the Restionaceae are now considered, it will be seen in table 1 that their perforation plates are much less specialized than those in the Gramineae. In the roots, scalariform plates are much more numerous, and such plates become progressively more common upward in the plant until in the leaves (usually reduced to sheaths) 27.3 per cent of the species have scalariform plates exclusively.

Study of species in the Juncaceae and Cyperaceae reveals variations of a nature comparable to that in the Restionaceae; wide variations occur from species to species in some organs, less wide in others.

The occurrence of the variations being discussed is important in terms of the number of genera — or species — which should be examined before generalizations about the families can be confidently made. Where variations are within a relatively narrow range, as in the Gramineae, a smaller proportionate number of known genera should give a clear picture of specialization within the family. On the contrary, where variations occur over a relatively wide range, a greater proportion of the known genera should be examined. It is clear therefore that in the Juncaceae and Restionaceae more genera should be examined before a clear statment of their average specialization can be made. It is noteworthy, however, that the levels of evolution of the vessel within the various organs of these two families comply with the generalization made by the present author (Cheadle, 1943b, p. 489) that phylogenetically the root leads in specialization and is followed in turn by stem, inflorescence axis, and leaf. Incidentally, Gilg's (1891) and Solereder and Meyer's (1929) reports on vessels in the stems of at least 20 species in 15 genera of the Restionaceae,[1] show that in these species the variation is about equal to that found by the present writer in his material.

Table 2 is designed to demonstrate the most primitive condition of vessels, as they have been categorized in terms of perforation plates for this paper, in the various organs of the four families under discussion. This information focuses attention on the most primitive conditions of vessels in each organ of each family which must be considered when the taxonomic use of specialization of vessels is described under the section on Discussion and Conclusions. It can be understood from a glance at table 2, for example, that the most primitive category of specialization of vessels discovered thus far in the roots of any species of Gramineae is never more primitive than it is in the same organ of any species examined to date in the other families. In the remainder of the plant in the grasses, the most

[1] Fahn (1954), in a paper seen by the present author after completion of the manuscript and figures for this paper, reported tracheids in the leaf sheath of *Hypolaena fastigiata* R. Br. and "probably" tracheids in the leaf sheath of *Leptocarpus tenax* R. Br. Neither species is considered in this paper, but others in these genera are. Because bundles in leaf sheaths in the Restionaceae are probably the remnants of smaller bundles or bundle ends of typical foliage leaves, the occurrence of only tracheids does not invalidate conclusions drawn in the present paper, which are based on tracheary elements in the larger bundles.

TABLE 2.

THE MOST PRIMITIVE CATEGORIES OF VESSELS PRESENT IN THE EARLY (E) AND LATE (L) METAXYLEM OF VARIOUS ORGANS IN THE FAMILIES

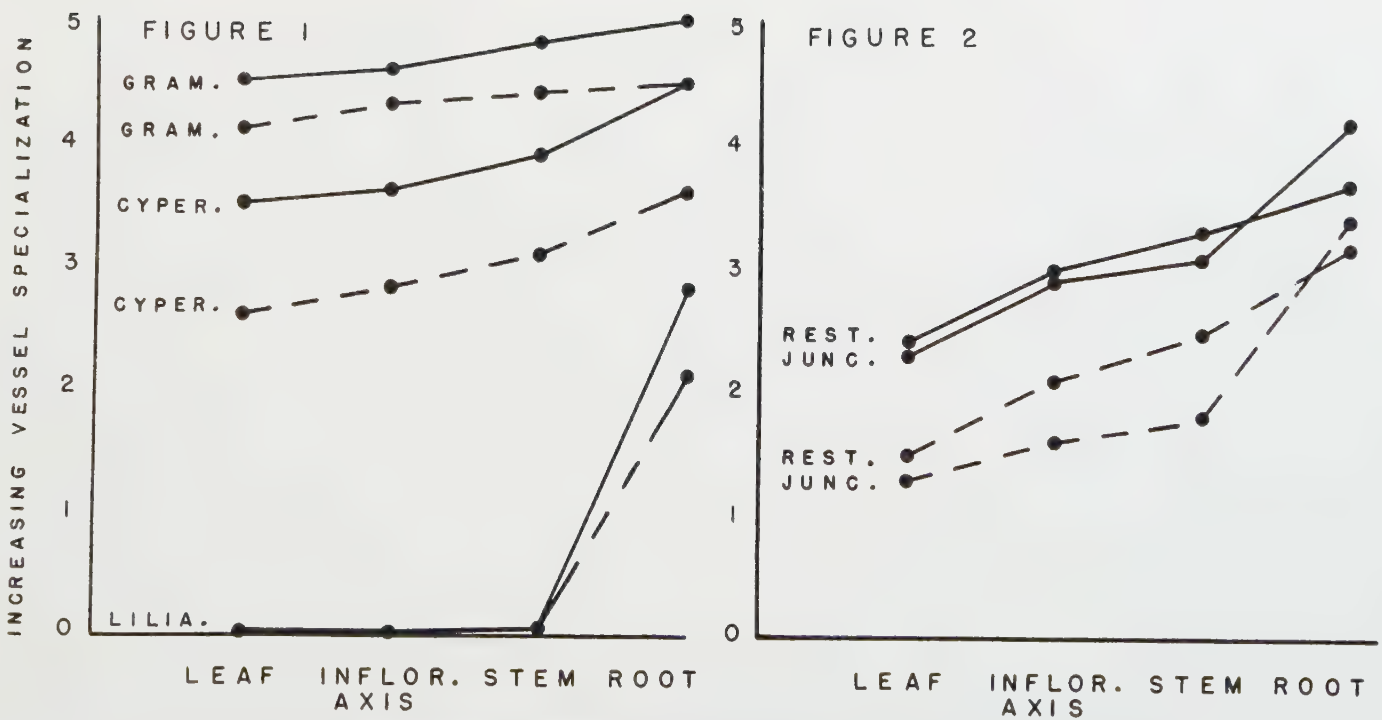
	Perforation Plates									
	Simple only		Mostly simple		Simple and scalariform		Mostly scalariform		Scalariform only	
	E	L	E	L	E	L	E	L	E	L
Roots										
Gramineae				x	x[1]					
Cyperaceae				x	x					
Juncaceae							x	x		
Restionaceae						x	x			
Stems										
Gramineae			x	x						
Cyperaceae									x	x
Juncaceae								x	x	
Restionaceae									x	x
Inflorescence Axes										
Gramineae			x	x						
Cyperaceae									x	x
Juncaceae								x	x	
Restionaceae									x	x
Leaves										
Gramineae					x[2]	x[2]				
Cyperaceae									x	x
Juncaceae									x	x
Restionaceae									x	x

[1] In *Zizania aquatica* L. only.
[2] *Pleuropogon californicus* (Nees) Benth. ex. Vasey only.

primitive categories of vessels are at a much higher level of specialization than are similar categories in the remaining families. It is noteworthy that species have been found in the Cyperaceae with exclusively scalariform plates in the shoot system even though the most primitive condition for perforation plates is at a relatively high level in the roots. The significance of the information in table 2 will be brought out in the section on Discussion and Conclusions.

B. RELATIVE AVERAGE SPECIALIZATION OF VESSELS IN THE VARIOUS ORGANS OF THE FOUR FAMILIES

Text-figures 1–3 represent an attempt to give in simplified form the relative average specialization of vessels in the four families, with the Liliaceae added for comparison. To make this type of representation possible, the categories of perforation plates in the five columns of table



FIGS. 1–2. Specialization of vessels in early (dashed line — roots only in Liliaceae) and late (solid lines) metaxylem in various organs of Gramineae, Cyperaceae, Juncaceae, Restionaceae, and Liliaceae. (Use of numbers described in text.)

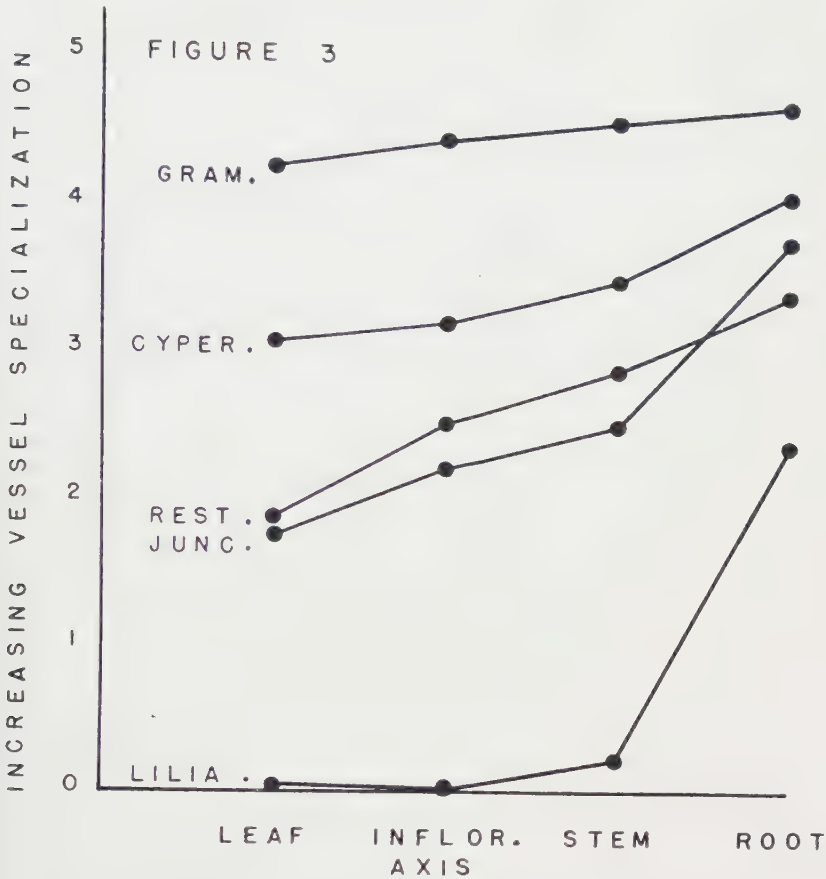


FIG. 3. Specialization of vessels in entire metaxylem in various organs of Gramineae, Cyperaceae, Juncaceae, Restionaceae, and Liliaceae. (Use of numbers described in text.)

1 have been arbitrarily assigned the following numerical values of specialization: scalariform only — 1, mostly scalariform — 2, simple and scalariform — 3, mostly simple — 4, simple only — 5. To make possible the inclusion of the Liliaceae, whose species generally have only tracheids in the shoot system, tracheids are valued at 0. Numerical values for each organ in every species were assigned to vessels or tracheids in both early and late metaxylem. These numbers were then averaged and the values thus obtained were used in making the graphical representation of specialization. Values for vessels in early and late metaxylem were averaged separately for text-figures 1 and 2. (The differences between these areas were so small in the shoot system of the Liliaceae that the early metaxylem is shown only for roots.) The average for all metaxylem in text-figure 3

was obtained by simply averaging the averages for early and late metaxylem. Lines are drawn from organ to organ in each family only to provide easier reference to values for such organs in the same family. Average values for all organs in one family were not used because of the wide differences, except for the Gramineae, usually shown in specialization of vessels in various organs of the same family.

This representation of the data makes it possible to note at a glance the average specialization of the vessels throughout the various organs of the plant in a given family and to compare the families organ by organ.

Two important features in text-figures 1 and 2 need discussion. One conclusion to be drawn from text-figures 1 and 2 is that in every organ the late metaxylem, on the average, is more highly specialized in terms of perforation plates than is the early metaxylem. It is obvious therefore that differences in specialization of vessels between any two families should not be measured between the early metaxylem in one family and the late metaxylem in the other. Such differences can be shown realistically, however, by comparing data from either early or late metaxylem or averages of the data from both late and early metaxylem. A second point of importance to be drawn from a study of text-figures 1 and 2 is that, within the same family, the metaxylem on the average differs from organ to organ in degrees of specialization, as measured by types of perforation plates present. As pointed out earlier, the root leads in specialization of vessels and is followed by stem, inflorescence axis, and leaf. Thus, for the most meaningful comparison of families, the average condition should be compared organ by organ. It follows that information in the literature on vessels which does not provide accurate observations in terms of organs, and of early and late metaxylem within the organs, cannot be used for studies of the present type, because such information is apt to be misleading in any event and may be useless when differences between families are small.

Several aspects of text-figure 3, which represents the average specialization of vessels in the metaxylem as a whole in various organs of the families, should be emphasized. (1) The high degree of specialization of the grasses is evident. Specialization of vessels in the leaves of the Gramineae, for example, has reached a point higher than that for vessels even in the roots of the Cyperaceae, in specialization the nearest rival to the Gramineae among the families represented in the figure. (2) Vessels have reached about the same level of specialization in the Juncaceae and Restionaceae, although those in the roots of the Juncaceae seem notably more specialized than those in the remainder of the plant in that family. (3) Vessels in the roots of the species of Liliaceae examined have reached a fairly high stage of specialization on the average, in spite of the fact that vessels are uncommon (and invariably primitive) throughout the shoot system. Variation in specialization of perforation plates in the roots of various species in the Liliaceae, however, runs through all gradations from the occurrence of (a) extremely primitive scalariform plates in the late metaxylem together with tracheids in the earliest metaxylem, to (b)

simple plates throughout the metaxylem. (4) Average specialization of vessels in the roots of the Liliaceae has surpassed that of vessels in leaves of the Juncaceae and Restionaceae and is comparable to that in both the inflorescence axes and stems of the Juncaceae and that in the inflorescence axes of the Restionaceae. (5) Organ for organ, the averages of specialization of vessels in the Juncaceae, Restionaceae, Cyperaceae, and Gramineae are above those of vessels in the Liliaceae. The greatest differences usually lie in comparisons of the leaves, the least differences reside in comparisons of the roots. (6) On the basis of increasing specialization of vessels, organ for organ, the families fall into the sequence Liliaceae, Juncaceae and Restionaceae, Cyperaceae, and Gramineae.

It is obvious from a consideration of text-figure 3 that some of the families differ markedly from one another in the average specialization of vessels and that others differ very little. The possible taxonomic use to which these differences can be put will be discussed in the next section.

DISCUSSION AND CONCLUSIONS

In dealing with data that are susceptible of interpretation in the form of salient trends of phylogenetic change, there is always the possibility that the data are too few or too unrepresentative to form a reliable source of information. One is constantly haunted, for example, by the possibility that accumulation of new data from larger numbers of species or from species from additional geographical areas over the world, or from additional environmental niches, might make necessary a substantial revision of our conclusions about the principal trends of evolution of vessels, even though such conclusions were induced originally from a considerable body of evidence. The present author has met this compulsion for additional evidence by continually adding to the body of data available for study of vessels. For example, although not all are included in the present paper, data from an additional 36 species of 25 genera in 7 families (one heretofore unavailable) have been considered since the last publication (Cheadle, 1953, p. 38) on vessels in the Monocotyledoneae. The total representation of the Monocotyledoneae is now 428 species in 274 genera of 42 families (as defined by Hutchinson, 1934). From all the additional 108 species (including those in 7 new families) that have been examined since the original publication on the origin and trends of specialization of vessels in the monocotyledons (Cheadle, 1943a), there has not come a single new observation to contradict the general statements made in that paper concerning phylogenetic changes in tracheary elements. This does not mean, of course, that such facts may not yet be discovered as a wider and wider representation of monocotyledons is subjected to study. Nevertheless, it becomes increasingly evident that the origin and specialization of vessels represent a series of evolutionary changes of perhaps unparalleled clarity in the monocotyledons (and in vascular plants generally, Bailey, 1953, p. 6).

In spite of the clarity with which the evolutionary development of

vessels in the monocotyledons can be described, the use of specialization of vessels to the taxonomist is limited. For example, within the Gramineae, the range of variation in specialization is so small that reliable measures of the differences in many instances are hardly possible and in any event would be impractical to ascertain for use in typical taxonomic descriptions. It can be reported, on the other hand, that only two (*Cladium mariscoides* (Muhl.) Torr. and *C. jamaicense* Crantz, as named in Fernald, 1950, p. 290) of the 36 species examined in the Cyperaceae have scalariform plates exclusively throughout the shoot system. Further investigation may prove that these species could be set apart, for purposes of identification, from the remaining members of the family on this basis alone. For the most part, however, information on vessels in terms of such use needs to be much more complete. Yet even in the absence of a more complete record, there seems to be justification for employment of tracheary elements as one of the factors in the determination of phylogenetic relationships of taxa within the monocotyledons. In some situations, the taxa may be species, in others genera, or tribes, or families, or even orders; the possible latitude can be determined only by further investigations.

If it is granted that tracheary elements are possible tools for use in developing phylogenetic relationships of members of the Monocotyledoneae, it should also be made clear that there are specific pitfalls to be wary of and a necessary body of knowledge to be acquainted with. Some of these matters were mentioned in the preceding section on OBSERVATIONS, but they are also included here for emphasis. (1) Tracheids are more primitive than any vessels. (2) Although scalariform perforation plates of any type are more primitive than simple perforation plates, those with few bars are more specialized than those with many bars. (3) Where readily identifiable variation occurs throughout the plant, taxa must be compared organ for organ. For example, vessels in roots of some species may have exclusively simple perforation plates before vessels appear in any part of the shoot system (*Aloe*), or in a given species specialization of vessels may be at the same low level (*Pandanus*) or at the same high level (many species of grasses) throughout the plant. (4) There is no real evidence of reversibility in the phylogenetic series from tracheids to highly specialized vessels, particularly as measured by the perforation plate. One possibility of misinterpreting observations as evidence for such reversibility in tracheary elements concerns congested areas in the plant, such as nodes, or slow growing rhizomes. Crowding of tracheary elements in congested areas may appear to reduce numbers of bars in scalariform plates and thus to illustrate phylogenetic reversibility. When such areas are properly evaluated in terms of a broad sampling, however, it will be found that physical crowding simply prevents the formation of elongate end walls, either by tracheids or vessel members, and thus large numbers of bars do not occur in perforation plates in such areas whether or not they occur elsewhere in the plant. The small number of bars can not be considered as reduction, for large numbers were never present in such areas; that is, the evolutionary series starts with short elements (tracheids)

in the first place and long end walls were never present in the phylogenetic change to vessels. As a matter of fact, lateral conduction may be emphasized in such congested areas, for perforation plates frequently occur on the lateral walls of vessel members. (5) It must be emphasized, as Bailey (1944, p. 425) has done, that specialization of vessels is chiefly reliable as evidence in "negations" of putative phylogenetic relationships. This point of view is based (a) on the conclusion that specialization of vessels is irreversible and (b) on the assumption that no other evolutionary series is so well founded on observable facts in extant or extinct plants. An illustration of the application of this approach to phylogenetic relationships is negation of the possibility that the Liliaceae arose from any extant members of the Butomaceae having simple perforation plates in their roots. Such an origin actually may seem reasonable in relation to the gynoecium, for example, but there are species in the Liliaceae having the most primitive types of scalariform plates on vessel members in the late metaxylem of the roots which negate such an origin for the Liliaceae. (7) From a positive point of view, specialization of vessels can offer only suggested relationships, which must be borne out by the weight of other evidence. In this connection, one must not overlook the possibility that even though in a family vessels may have reached a high average degree of specialization, the occurrence in it of species with very primitive vessels may preclude a possible origin of this family from another family in which the average specialization of vessels is relatively low, but in which no species with really primitive vessels occur. An illustration of this statement occurs in point 3 in the following paragraph.

Keeping in mind the enumerated statements just made, the observations noted earlier in the paper, table 2, and text-figure 3, some examples of the use of specialization of vessels in determining possible phylogenetic relationships as they concern the Gramineae, Cyperaceae, Juncaceae, Restionaceae, and Liliaceae, as now known, can be enumerated. It should be emphasized that especially points 3, 4, and 5 below are tentative statements and that their validity must be checked by study of more representatives of the families Restionaceae and Juncaceae. One now knows, however, what critical stages of specialization of vessels to look for in any new materials of these families. (1) The Gramineae, as known from investigations up to date, could not have given rise to any of the other four families represented in text-figure 3, because vessels in all species of the grasses have already reached too high a degree of specialization. (2) The general average of specialization of vessels in the Cyperaceae is rather high, but there are two species [*Cladium mariscoides* (Muhl.) Torr. and *C. jamaicense* Crantz] whose vessels in the shoot systems are as primitive, or more primitive, than those in species of the Restionaceae or Juncaceae. In the roots of these two species, however, the vessels are more specialized than are vessels in the same organs in some members of the Restionaceae or Juncaceae. The Cyperaceae, on the basis of present knowledge, thus can not serve as a source of the Juncaceae or Restionaceae. (3) No species examined to date in the Juncaceae has vessels quite primitive enough

throughout the plant to permit use of the Juncaceae as the source of the most primitive members of the Cyperaceae or of the Restionaceae. (4) Vessels of one species in the Restionaceae (*Lyginia* sp.) are primitive enough to make possible the origin of the most primitive members of the Cyperaceae from the Restionaceae. (5) In spite of the primitive vessels throughout the shoot system of one species in the Restionaceae (*Lyginia* sp.), vessels in the roots of that species are not primitive enough to make possible the origin of the most primitive members of the Juncaceae from the Restionaceae. (6) Numerous species in the Cyperaceae, Restionaceae, and Juncaceae have vessels throughout the plant primitive enough to make possible the origin of the most primitive members of the Gramineae from any of these families. (7) There is no possibility of deriving the Liliaceae from the Juncaceae, Cyperaceae, Restionaceae, or Gramineae because all species in these four families have vessels throughout the plant and most species in the Liliaceae lack vessels throughout the shoot system. (8) Although a few species in the Liliaceae have vessels throughout the plant, all these have exclusively the most primitive types of vessels in the shoot system. Vessels in the shoot system of the Juncaceae, often thought to be closely related to and derived from the Liliaceae (Hutchinson, 1934, p. 187) are more specialized. This derivation of the Juncaceae, even from some of those species in the Liliaceae having vessels in the shoot system, therefore is supported by the relative specialization of vessels in the two families. (9) Although the Juncaceae may have been derived from the Liliaceae, as understood by Hutchinson (1934), vessels in the roots of a number of species of the Liliaceae (e.g. all investigated species of *Aloe*, *Asparagus*, *Johnsonia*, *Paradesia*) preclude involvement of these species in this derivation, because these vessels are already more highly specialized than those that occur in the roots of some species in the Juncaceae. (10) On the same basis as noted above for the Juncaceae, except for minor differences in detail, the Cyperaceae, Restionaceae, and Gramineae could all have been derived from the Liliaceae.

These enumerated statements provide examples of the possible uses of the absence of, and when present the specialization of, vessels in the various organs of the plants in the Monocotyledoneae. It is obvious that the more complete the representation of examined species the more useful the information on vessels will be. Additional information accordingly will be made available from time to time as it is collected. Meanwhile, the author's more detailed information on species already studied is available to anyone who has good use for it.

SUMMARY

1. The average specialization of vessels in the metaxylem of the Gramineae, Cyperaceae, Juncaceae, and Restionaceae is described; that of the Liliaceae is considered for contrast. On the basis of specialization of vessels, the Gramineae rank highest and are followed in turn by the Cyperaceae, Juncaceae and Restionaceae, and Liliaceae.

2. The most primitive condition found in any species in each family is given, together with the variation of specialization of vessels in every organ in all families.

3. The clarity and the irreversibility of the phylogenetic trends in the origin and specialization of vessels in the Monocotyledoneae are discussed in terms of using variations in specialization of vessels to help determine certain possibilities of phylogenetic relationships of the families described.

4. After limitations and necessary qualifications in the use of specialization of vessels in phylogenetic studies of taxa are reviewed, suggested examples of this use are provided. Among the more important of these, in the light of our present knowledge, are: The Gramineae can not be the source of any of the remaining families, but the converse is true. The Juncaceae and Restionaceae can not be derived from the Cyperaceae. The Cyperaceae can be derived from the Restionaceae, but not from the Juncaceae, in so far as they have now been examined. On the basis of available evidence, the Juncaceae and Restionaceae can not be derived from each other. The two preceding statements are actually tentative, and further material is needed to test their validity. The Liliaceae can not be derived from any of the other four families, but all four can be derived from the Liliaceae; yet there are many existing species in the Liliaceae which could not have taken part in this derivation.

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EXPLANATION OF PLATE I

FIG. 1.?*Pleioblastus argenteostriatus* (Regel) Nakai (Gramineae). Transverse section of vascular bundle from stem, showing two large vessels (LM) of late metaxylem and smaller vessels (EM) in early metaxylem. × 131.

FIG. 2.?*Pleioblastus argenteostriatus* (Regel) Nakai (Gramineae). Transverse section of portion of root, depicting vessels in late metaxylem (LM) and vessels in early metaxylem (EM), together with sieve tubes (S) not in the customary phloem position. The tiny, thin-walled cells (PH) represent the phloem strands in typical peripheral position. × 148.

FIG. 3. *Cyperus filiculmis* Vahl (Cyperaceae). Transverse section of bundle from stem, showing two vessels in late metaxylem (LM) and the early metaxylem area (arrow from EM). The letters EM are actually placed in the protoxylem lacuna. × 420.

FIG. 4. *Cladium mariscoides* (Muhl.) Torr. (Cyperaceae). Longitudinal section of stem, showing vessel members with end walls in section. Relatively small number of perforations occurs in the long end walls of vessel members. Imperforate pits occur in the upper half of the end walls. × 420.

FIG. 5. *Cladium mariscoides* (Muhl.) Torr. (Cyperaceae). From same section as in Fig. 4, but with vessel members having longer end walls and greater number of perforations. Note thinner bars in center of end walls. × 420.

FIG. 6. *Carex lurida* Wahlenb. (Cyperaceae). Longitudinal section of stem, showing vessel members with their oblique end walls cut on bias. Branching of bars and variation in size of perforations (areas between bars) are evident. × 258.

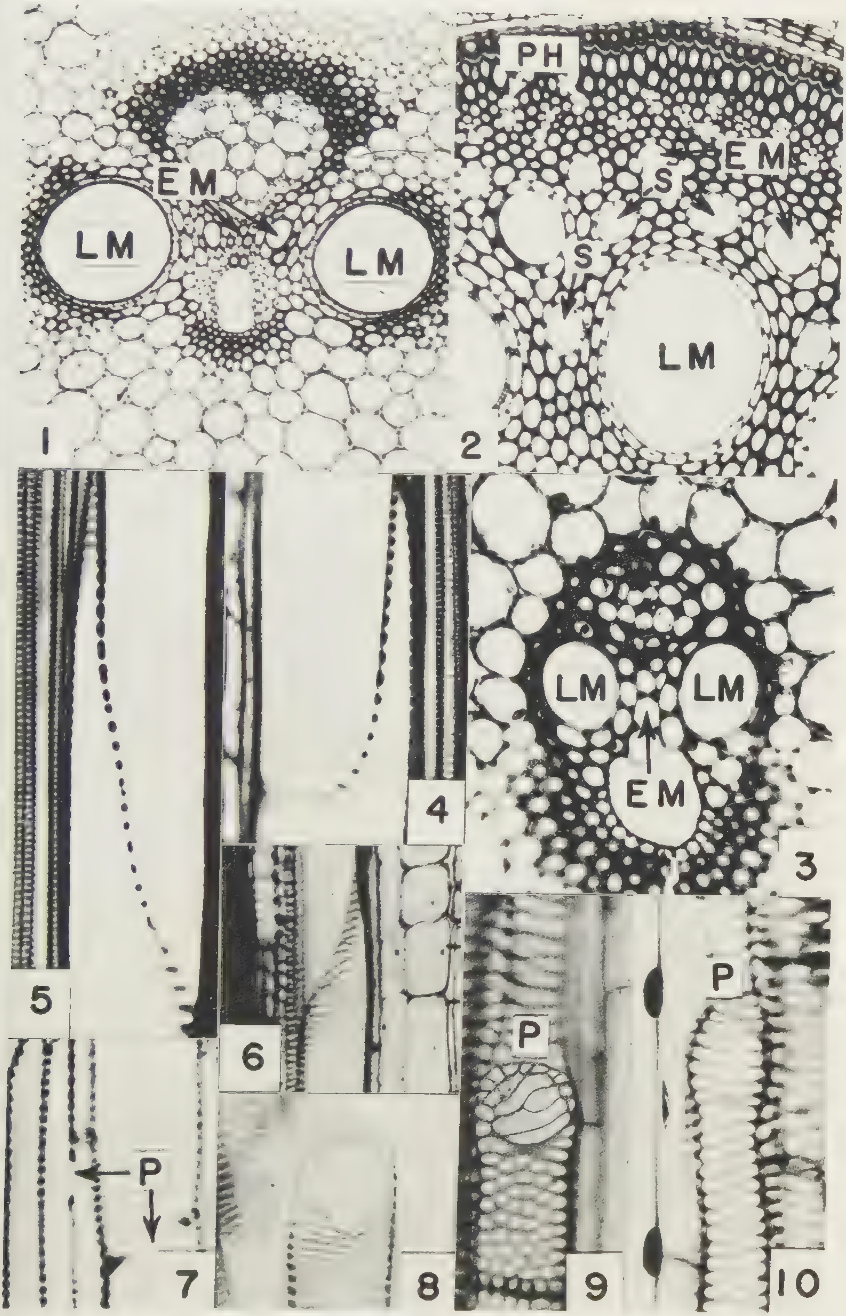
FIG. 7. *Arundo donax* L. (Gramineae). Longitudinal section of bundle from

leaf, illustrating simple perforation (P) in end walls of large vessel members in late metaxylem and one on side walls of small vessel members in early metaxylem. × 510.

FIG. 8. *Carex intumescens* Rudge (Cyperaceae). Separated vessel element from stem, illustrating simple perforation with remnant of bar discernible at right. Remnants of varying number frequently occur in perforation plates of vessel members in some species of Cyperaceae, Juncaceae, and Restionaceae. × 578.

FIG. 9. *Zea mays* L. (Gramineae). Longitudinal section of stem, showing small branched scalariform plate (retouched) — just below P — in side wall of vessel member in early metaxylem. Only large pits are perforate; the smaller ones (sides and top) are imperforate. × 510.

FIG. 10. *Zea mays* L. (Gramineae). From same section as in FIG. 9, but vessel member with more typical scalariform plate (just below P) on lower two-thirds of end wall. Reticulate wall thickening evident on side wall in face view. × 510.



Cheadle, Specialization of Vessels

THE POLLEN MORPHOLOGY OF SEVERAL GENERA EXCLUDED FROM THE FAMILY ICACINACEAE

A. Orville Dahl

With one plate

The Family Icacinaceae Miers, has been investigated in detail in recent years with respect to secondary xylem (*cf.* Bailey & Howard, 1) and pollen (Dahl, *2*). During the analysis of pollen of all of the available genera, pollen material of several genera either assigned with some question to the Icacinaceae or believed to have possible alliances with this family were examined. These include the following: *Pseudobotrys, Lophopyxis, Pteleocarpa, Metteniusa* (*Aveledoa*), *Peripterygium* and *Trichadenia*. Some authors have recommended that some of these genera be excluded from the Icacinaceae (*cf.* Howard, 5 and 6 and Sleumer, 7). In view of these circumstances, a summary of the pollen characters occurring in this material seems in order in terms of possible clues concerning the natural relationships of the entities involved. Reference to detailed data and figures will make it evident that each of the species is distinct from the standpoint of pollen morphology.

To Professor I. W. Bailey, I am very grateful for most of the pollen specimens examined. Dr. H. Sleumer kindly called my attention to specimens of *Metteniusa tessmanniana* in the herbarium of the New York Botanical Gardens. I am indebted to Dr. David Keck for the recent loan of this material.

All of the observations as herein reported are based upon pollen derived from herbarium specimens. (The letter A refers to specimens from the Herbarium of the Arnold Arboretum while NY refers to those from the New York Botanical Gardens.) The preparational procedure for the lactic acid mounts has been previously summarized (*2*). The figures, while completed in semi-diagrammatic form, are based upon drawings of individual grains made with camera lucida. An apochromatic objective (90X, N.A. 1.3) and compensating oculars (12X) were used for observation. Miss Wilma Monserud has kindly prepared the illustrations. Data on pollen grain size refer, respectively, to length and diameter.

The chief diagnostic characters of the pollen of the seven entities analyzed are given below. Most of the descriptive terms used follow the applications presented by Faegri and Iversen (4) and Erdtman (3).

1. PSEUDOBOTRYS Moes.

Pollen grains oblate; triaperturate; large, bulging elliptical apertures; possibly tricolporate since a depression or fold extends from the aperture

in direction of the poles; exine foveate to foveolate; in the immediate vicinity of the aperture, a region of exine (to the radial extent of ca. 3–4.5 μ) is very thin thereby contributing to an ascending or protruding character of the aperture.

Pseudobotrys cauliflora (Pulle) Sleumer. — FIGS. 1, 1A. — 54.3 × 62.4 μ. — *Brass 7005* (A).

2. LOPHOPYXIS HOOK. F

Grains ellipsoidal; tricolporate; furrows constricted at the equator, obscuring the germ pore; pore circular to somewhat oblong (long axis parallel to the equator) in outline; exine conspicuously foveate with many of the foveae of elliptical outline.

Lophopyxis pentaptera Engl. — FIGS. 2, 2A. — 38.9 × 30.2 μ. — Collector unknown *1775* (A).

3. PTELEOCARPA OLIV.

Grains spheroidal to ellipsoidal; tricolporate as in *Lophopyxis*; furrows constricted over the germ pore; exine foveate, very much as in *Lophopyxis*.

Pteleocarpa malaccensis Oliv. — 37.0 × 32.0 μ. — *Maingay* (A).

4. METTENIUSA KARSTEN (INCLUDING *Aveledoa* PITTIER)

Pollen grains ellipsoidal; triangular in polar view because of the domed or vestibulate character of the germ pore region; tricolporate with distinct, usually bulging germ pores of more or less rectangular outline (see FIGS. 3A and 4A); frequently small granules of exine occur on the pore and furrow membranes; exine, while almost smooth in outline in its general effect, possesses a distinctive complexity in its fine structure; in polar regions the exine is foveolate-foveate; as one proceeds to equatorial regions there is a transition to a delicately rugulate character that finally is supplanted by a finely areolate-verrucate surface, particularly in the region of the equator; in the vicinity of the furrows the exine is foveolate. It should be noted in FIGS. 3 through 4A that the various exine structures (foveae and areolae) are presented in outline only because of their small dimensions.

Metteniusa nucifera (Pittier) Sleumer. — FIGS. 3, 3A. — 42.0 × 40.8 μ. — *A. Jahn 1202*, CO-TYPE (NY).

As in the following species the furrows are well-defined with minute irregularities in the margin. Occasionally the furrow is constricted over the germ pore (FIG. 3A).

Metteniusa tessmanniana (Sleumer) Sleumer. — FIGS. 4, 4A. — 42.8 × 39.7 μ. — *G. Tessmann* probably *4042*, TYPE (NY); *G. Tessmann 4235* (NY).

Within the genus, the areolate-verrucate character of the exine was most conspicuously developed in *4235*.

5. PERIPTERYGIUM Hassk. (? *Cardiopteris* Wall.)

Grains oblate; triangular in polar view; tricolporate; furrows narrowly tapering and extending almost to the more or less flattened poles; exine foveolate with the exception of the polar regions; foveolae are noticeably aligned radially giving a locally striate effect.

Peripterygium moluccanum (Blume) Sleumer. — 10.7 × 16.3 μ — Collector unknown *38579* (A).

6. TRICHADENIA Thw.

Grains spheroidal; tricolporate with long, tapering well-differentiated furrows; in direction of the poles, the circular germ pore is sometimes bounded by small club-shaped elements (pilae or clavae); exine conspicuously clavate; the pilae while polymorphic are largely dimorphic, *i.e.* of two size classes; the pilae of small size have a diameter (head) of ca. 0.7 μ while those of large size are 1.5 to 3.2 μ in diameter; the large elements are scattered over the surface imparting a striking warty appearance to the grain.

Trichadenia philippensis Merr. — 28.7 μ (diameter) — Phil. Bur. Sci. *2982* (A).

DISCUSSION

None of the pollen complexes described above agrees entirely with the highly distinctive patterns of pollen morphology occurring in a number of representatives within the family Icacinaceae. However less specialized foveate, triaperturate types of pollen occur in a number of species within the Icacinaceae. Such types are also found in a number of other families. Of the genera discussed here, only *Pseudobotrys* and *Lophopyxis* in their pollen morphology have some, but not marked, similarity to certain genera in the Icacinaceae. For example, *Leptaulus* has some features of *Pseudobotrys* while *Icacina*, *Lavigeria*, and *Leretia* exhibit some characters of *Lophopyxis*. However, the pollen morphology of the investigated preparations does not suggest their inclusion within the Icacinaceae as presently defined. Their affinities on the basis of pollen alone are, naturally, difficult to sense.

The placing of *Pteleocarpa* in the Boraginaceae is not supported by pollen morphology. Indeed it should be noted that pollen of the specimen labelled "*P. malaccensis* Oliv." is identical with that of *Lophopyxis*. Further, the suggestion that *Pteleocarpa* be assigned to *Peripterygium* is not verified by the pollen preparation. The genus *Lophopyxis* would appear to be its rightful assignment. It is relevant also to note that the range of pollen forms known to exist within the family Euphorbiaceae could include that of *Lophopyxis* (see Erdtman).

Peripterygium has been assigned to the family Peripterygiaceae. Slight

similarity in size and apertures of the pollen grains of *Peripterygium* to those of *Coula* in the Olacaceae has been reported by Erdtman (3).

The various affinities of *Metteniusa* cannot be stated here with certainty. Sleumer (7) assigns this genus to the tribe Icacineae of the Icacinaceae whereas Howard (5) suggests its exclusion from the family. While the pollen of *Metteniusa* possesses some of the wide-spread characters seen in many families including the genera within the Icacinaceae having Type A pollen (2), it does not agree in its distinctive complex of characters with any of the individual examples of pollen within the Icacinaceae. For example, the pollen of *Metteniusa* differs in number and structure of the germinal apertures from *Alsodeiopsis* and in structure of the germinal apertures from *Merrilliodendron*. It will follow that its inclusion within the family Icacinaceae would represent the addition of a new combination of pollen characters. Howard (5) has reported, also, that in a number of its floral (corolla, stamen, and pedicel) characters it finds no counterpart, in so far as presently known, within the family.

The suggestion that *Metteniusa* has alliance with the Convolvulaceae is not confirmed by pollen morphology. The pollen grains of *Maripa* and *Erycibe*, for example, are typically convolvulaceous and markedly differentiated from those of *Metteniusa*.

The assignment of *Metteniusa* to the family Boraginaceae near the genus *Cordia* seems inappropriate on the basis of pollen characters. *Cordia gerascanthus* L., for example, produces entirely distinct echinate, oblately spheroidal, tricolporate pollen grains.

Thus far, I have no pollen evidence clearly relating *Metteniusa* to the several pollen types included within the Olacaceae. An illustration of the distinctly different pollen form is seen in *Olax scandens* Roxb. and *O. triplinervis* Oliv. where the triporate pollen grains are psilate and oblate (see also Erdtman, 3, for other examples).

In its unusually distinctive pollen grain, *Trichadenia* is somewhat suggestive of *Ilex* in the Aquifoliaceae. However the marked dimorphism of the pilae in *Trichadenia* is not seen in *Ilex verticillata* Gray where the pilae or clavae are relatively more uniform and of small size. The inclusion of *Trichadenia* in the family Flacourtiaceae gains support from an examination of pollen of another genus in this family, namely in *Samyda grandiflora* Griseb. where the tricolporate pollen grains exhibit a suggestively similar warty surface.

From the above analysis of the available pollen material, one may conclude that a number of these entities do not exhibit typically icacinaceous pollen characters. Until a larger complex of various characters can be fitted together, the more or less detailed affinities of some of these genera largely remain a problem for the future. In a number of instances, however, the data on pollen suggest the possible relationships which are worthy of serious consideration.

SUMMARY

1. Descriptions are provided summarizing the pollen morphology of species assigned to the genera: — *Pseudobotrys, Lophopyxis, Pteleocarpa, Metteniusa, Peripterygium*, and *Trichadenia.*

2. The possible affinities of these entities are discussed in terms of known patterns of pollen form.

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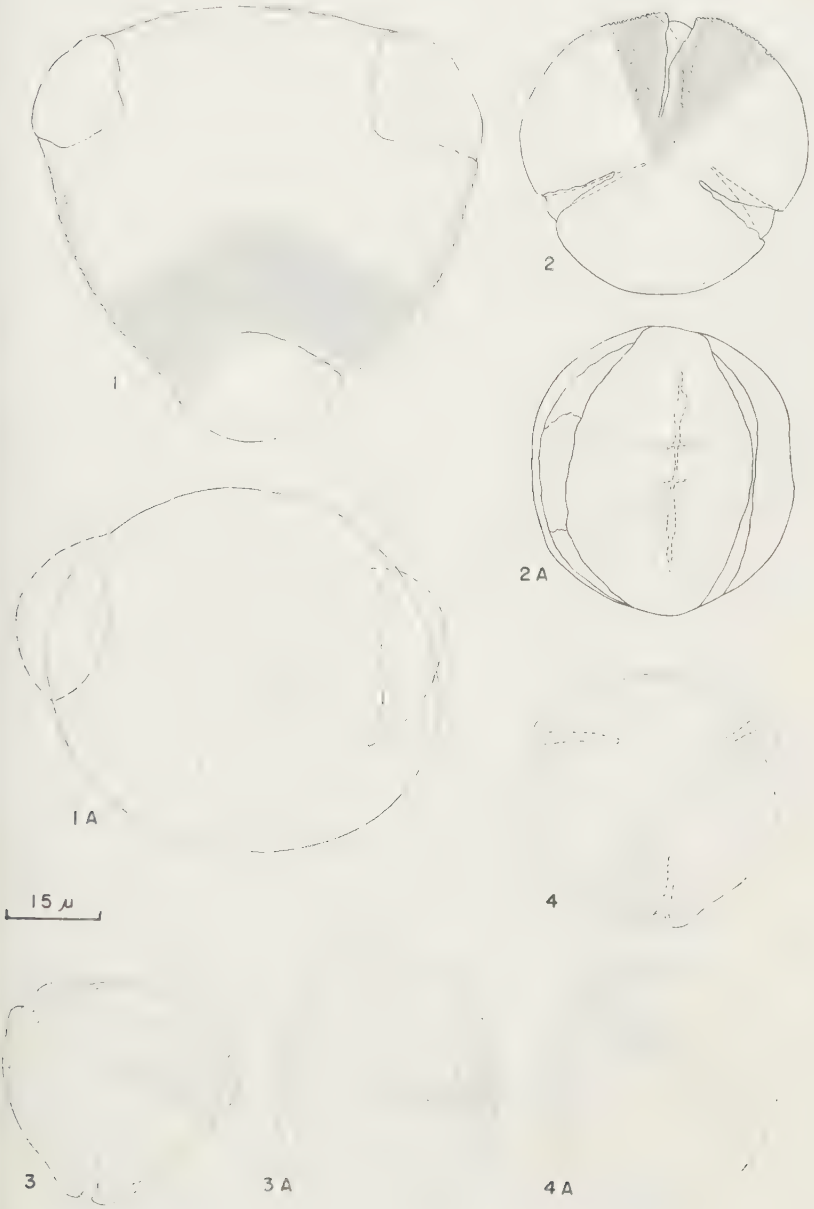
DESCRIPTION OF PLATE I

The figures are based upon camera lucida drawings of unstained pollen mounted in lactic acid. The original drawings represent a magnification of ca. 1600–1700 ×. In the present publication the illustrations are reduced by approximately one-half.

Fig. 1. *Pseudobotrys cauliflora* (*Brass 7005*, A), polar view. Fig. 1A. ditto, equatorial view. Fig. 2. *Lophopyxis pentaptera* (collector unknown *1775*, A), polar view. Fig. 2A. ditto, equatorial view. Fig. 3. *Metteniusa nucifera* (*A. Jahn 1202*, co-type, NY), polar view. Fig. 3A. ditto, equatorial view. Fig. 4. *Mettensiusa tessmanniana* (*G. Tessmann* probably *4042*, type, NY), polar view. Fig. 4A. ditto, equatorial view.



Dahl, Pollen Morphology

THE SEED AND GINKGO

ARTHUR J. EAMES

WHAT IS A SEED? When is a "seed" not a seed? For the seed — as for many other plant structures — no technical definition rigidly applicable is possible. The evolutionary history of this morphologically complex structure seems now fairly well known. The study of paleozoic reproductive structures of seed type has emphasized the fact that certain stages in the evolution of the seed cannot readily be set apart from others as "the seed." (Whether the seed arose through heterospory or through homospory is still in question and need not enter this discussion.) The various definitions and uses of the term seed cover every stage in the elaboration of the seed from fertilization to the presence of a mature, dormant embryo, and extend downward in the evolutionary series to cover the many types of paleozoic "seeds" which show no evidence of an embryo. In most botanical and popular definitions it is implied that a seed must have a dormant embryo. A frequently used basis of definition is expressed simply by Chadefaud (1944): "After fertilization the ovule becomes a seed." But in common usage for a long time, the fossil ovule-like reproductive structures of various taxa have been freely called seeds although they possess no embryos. Also, so-called seeds of *Ginkgo* and some cycads may have no embryo. Obviously, the term seed is loosely used. If it is to be used strictly, the line between ovule and seed must be drawn at fertilization. On this basis, it cannot be determined whether many fossil seed-like structures are ovules or seeds, and many complications of terminology, nomenclature, and classification arise. Nor can the plum-like "fruits" of *Ginkgo* that fall to the ground with the leaves be called with certainty, seeds or ovules; they may be either. In some conifers and some angiosperms, weeks, even months intervene between pollination and fertilization. What are the "ovules" to be called during the period when it is uncertain whether fertilization has occurred? Obviously, for convenience, the term seed must be used somewhat loosely, as are many other terms.

The use of the seed to characterize a natural group is now recognized as without value. The long-accepted "Spermatophyta" has disappeared from modern systems of classification because the possession of seeds brings together unrelated taxa, and the use of a group of other characters makes a more natural classification. Similarly, the "Gymnospermae" — characterized by the possession of *naked* seeds — also are now seen to be a highly unnatural group and the familiar and long useful term will soon be discarded in natural classifications.

It has been emphasized many times in recent years that natural relationships can safely be established only on groups of characters, not on a single character. Yet new classifications continue to be based on the seed.

Emberger (1942) has proposed the new group Praephanerogamae. On the basis that phanerogams differ from the Pteridophytes in the possession of an ovule (a megasporangium enclosed in tissues of the sporophyll), an intermediate group, the prephanerogams, stands apart as a taxon in which ovules are present but are shed without an embryo. The pteridosperms and cordaites form this group, as originally proposed. Favre-Duchartre (1945) added *Ginkgo* to the prephanerogams — rather illogically because, as he says, the falling ovules contain "des archégones encore vièrges ou de jeunes embryons indifferenciées . . ." He calls *Ginkgo* the only living prephanerogam, yet describes it as having the distinguishing characters of both Emberger's prephanerogams and phanerogams. Emberger (1944), in his extensive textbook, maintains the Ginkgoales as phanerogams, stating that fertilization occurs only after ovules fall, though citing Favre-Duchartre. In his later description of the new group (1949), he includes both the Ginkgoales and the Cycadales "because of the published researches and observations of Favre-Duchartre (1943), Chadefaud (1944), Mangenot (1945), and Martens (1947)." "These [gymnosperms] are the last survivors of this group."

Florin (1950) has critically discussed Emberger's segregation of prephanerogams, and Martens (1951), in a review of the nature of the seed and its evolutionary history, has also discussed this new grouping of seed plants. Florin concludes that Emberger's classification "is artificial and should be rejected." This paper is written in support of Florin's view by emphasis on the importance of *Ginkgo* as a plant transitional between the prephanerogams and phanerogams of Emberger. It is written to bring attention also to the largely overlooked fact that *Ginkgo*, in its variations in time and place of fertilization, demonstrates an important step in the evolution of the seed — that of the transfer of fertilization from the ground to the mother sporophyte — and it adds information from the experience of the writer.

Unfortunately, details of the time and place of fertilization in *Ginkgo*, though known in a general way since the early studies of Strasburger (1872), have largely been overlooked or regarded as unimportant. But they are of much importance from the standpoint of such clasifications as that of Emberger. Strasburger stated that fertilization may occur in ovules on the tree or after they have fallen, even "in a warm room," and that embryo development is the same wherever fertilization occurs. That fertilization may occur in ripe ovules on the ground as well as in unripe ovules on the tree has been largely disregarded, especially in this country. Van Tieghem (1884) states in his voluminous text that fertilization occurs in the ripe seed after its fall and that embryo development continues through the winter. Martens (1951) outlines the statements of various authors. Though Hirase (1894) at first described fertilization as on the tree, he later (1898) states that it occurs on the ground, though in some seeds on the tree. Ikeno (1901) states that fertilization occurs on the tree, that it may possibly occur on the ground, but that as far as he knows, "this has never been demonstrated." (Hirase also states that embryo

development may be completed on the tree or after falling. Apparently no other author has described full development of the embryo on the tree and Hirase's statement is probably in error.) Seward and Gowan (1900), in their memoire, apparently following Hirase, state that fertilization may occur before or after ovule fall. Strangely, neither Coulter and Chamberlain's textbook (1917) nor Chamberlain's much later text (1935) mentions place of fertilization. Chadefaud (1944) found that in Paris "ovules fall at the time of fertilization but also afterward." Studies made by the writer some years ago showed that in Ithaca, N. Y., fertilization may occur in late September in green ovules on the tree; in October in ripe ovules on the tree, or on the ground, even some days after they have fallen. The time of fertilization varied considerably in ovules on the same tree and among different trees in the same season and on the same tree in different seasons. Favre-Duchartre (1943) reported that fertilization may occur either just before, or after ovule fall, and that embryo development may continue to maturity even in the laboratory. Dangeard (1946) reported finding embryos in ovules on trees in mid-September in one year and in mid-August in another. Martens (1951) surmised that time of fertilization is climatically controlled. In the light of the writer's experience it seems more likely that it is related to age and time of pollination of the individual ovule. Fertilization in *Ginkgo* clearly may occur over a fairly long period and either on the tree or on the ground. The existence of this condition in a representative of a primitive seed-plant is important because it is probably an illustration of the ancient step in seed evolution when fertilization was transferred from the ground to the mother sporophyte. The survival of *Ginkgo* is, in one more way, most fortunate — as a demonstration of a critical step in the phylogenetic history of the seed.

Emberger's classification breaks down on the basis that *Ginkgo* falls in both of his groups. Further, Florin (1950) points out that Emberger's classification separates the Cordaitinae and Ginkgoinae from the Coniferae. And the growing opinion, substantiated by evidence from several fields, is that the Cordaitales, Ginkgoales, Coniferales, and Ephedrales form a natural group (Eames 1953).

DORMANCY IN PRIMITIVE SEEDS

Ginkgo, together with the cycads, illustrates another important step in the development of the seed, a step in the establishment of dormancy in the embryo. Though dormancy is often considered an essential character of the seed, the story of its development has received little attention. Dormancy of the embryo is an outstanding feature of the seeds of angiosperms and higher gymnosperms. The presence or absence of dormancy in the lower living gymnosperms — *Ginkgo* and the cycads — is rarely mentioned. In the lower fossil gymnosperms — pteridosperms and cordaites — the condition is unknown. Florin (1950) remarks "It appears probable that the ovules of the ancient gymnosperms always had a rest period, either after fertilization or after the shedding stage." He has also said

that the common absence of embryos in paleozoic seeds may well be explained "by the shedding of ovules rather than seeds." Arnold (1948) believes that paleozoic ovules were shed at a particular stage of maturity and that they entered a rest period at that time. (Arnold also suggests that the presence of a hard, durable integument enclosing the gametophyte may be evidence of dormancy in the ovules. But this condition is not necessarily evidence of a resting stage, for *Ginkgo* and some of the conifers, especially species of *Podocarpus*, have such histologically hard and tight layers before fertilization.) The writer agrees with Florin and Arnold that the ancient "seeds" without ovules are unfertilized ovules or seeds with early stages of embryos and that the absence of embryos suggests a resting period at the time of shedding or soon thereafter. Though *Ginkgo*, in its shedding of ovules and seeds with proembryos, shows in living plants the condition suggested for paleozoic plants, it does not give support to the existence of a rest period at this stage.

Neither *Ginkgo* nor the cycads show fixed dormancy at any stage; they do show induced dormancy, a dormancy induced by conditions unfavorable to continuous growth of the embryo or to germination if the embryo is mature. Such induced dormancy may well be a step to the fixed dormancy of other plants.

For *Ginkgo*, published statements imply embryo growth continuing until germination. Van Tieghem (1884) found embryo development through the winter. Hirase (1894) reports that the fall of the seed does not interrupt the growth of the embryo which continues until spring. Chamberlain (1935) states that embryo development continues in the laboratory or under stratification out-of-doors to germination in April; there is little or no dormancy, but development is more rapid in the laboratory. The writer followed embryo development in stratified seeds kept out-of-doors through the winter. (The seeds used were from a lot that had fallen and in which fertilization was occurring on the ground.) The studies showed that growth continued throughout the winter whenever temperatures were above freezing, and germination occurred in April without any apparent dormancy. Seeds held in warm places probably do complete embryonic growth and lie dormant until conditions are favorable for germination; here dormancy is induced.

In the cycads also, embryo growth is often stated or implied as continuing after the seed is shed but direct statements concerning dormancy are rare. Baird (1939) states that in *Macrozamia reidlei* growth continues in the seed for over 12 months without cessation before germination. The author has noted a similar condition in *Cycas circinalis*, with embryo development through several months. Chamberlain (1910), describing the embryogeny of *Dioon edule*, states that in this plant germination occurs without a dormant period but that seeds germinated after lying in a laboratory for over two years. Chamberlain later (1919) wrote "the cycad has no resting period, development being continuous from fertilization to old age and death."

Doubtless in both the cycads and *Ginkgo*, embryo development is con-

tinuous until germination under favorable climatic conditions, but dormancy may develop under conditions unfavorable to germination. Dormancy is not fixed but may be induced. These plants provide a step between continuous embryo-seedling development and the establishment of a dormant period at a determined stage in embryo development. In definition of the seed, no line can be drawn between the absence and the presence of dormancy in the embryo. It may be desirable — it is certainly convenient — to draw a line between the ovule and the seed, the presence of an embryo of any stage determining a seed. But it is a bit awkward to say that the *Ginkgo* tree sheds both ovules and seeds — that some of the fruit-like structures are ovules and others seeds without being able to point out which are which.

Summary. The definition of "seed" is discussed and the bearing of *Ginkgo* and its "seeds" on Emberger's segregation of Prephanerogams from Phanerogams. *Ginkgo*, in that fertilization takes place either on the tree or on the ground, falls in both of these groups, showing the classification to be artificial. This genus shows the important step, in the evolution of the seed, of the transfer of fertilization from the ground to the mother sporophyte. With the cycads it shows a step in the establishment of dormancy of the embryo in the seed.

The writer wishes to acknowledge his indebtedness to Florin and Martens on whose papers he has drawn freely in this discussion.

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SOME FACTORS IN POLLEN GERMINATION CALCIUM SALTS, DEXTROSE, DRYING

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IN HONOR OF IRVING W. BAILEY whose pioneer studies in cell structure, behavior and organization have been an inspiration, this paper is presented. It is a brief summary and discussion of experiments reported at the last three meetings of the A. I. B. S. but otherwise unpublished.

Pollen is notorious for the uncertainty of its behavior in germination and tube growth. Yet very little work has been done on the factors involved. A few studies have been made as part of more comprehensive programs. Bungenberg de Jong and Henneman (1934) in their work on cell models and neutral salts reported on pollen. Smith (1939) tested indole-3-acetic acid as a pollen germinant. Loo and Hwang (1944) included pollen in a series of papers on indole-3-acetic acid, manganese sulfate and colchicine as growth stimulants. Thomas, Gauch and Dugger, Jr. (1952) extended work on boron effects to include pollen, and O'Kelley (1954) used C-14 sugars to examine the rôle of carbohydrates in pollen growth. A brief summary of the literature has been made by Nitsch (1953) in his review of fruit growth.

In the present study of pollen physiology suggested by K. V. Thimann [1] in 1945, it soon became apparent that information on pollen germination requirements was incomplete. The essential tools of a reliable germinating technique and a growth medium were still lacking. To develop these it was necessary to determine the factors involved in pollen growth and their operation in terms of cell structure.

A preliminary survey of chemical stimulants and environmental factors was made in March to June, 1946–1949, on 28 species of the Texas Gulf Coast. None germinated satisfactorily in water, but, used separately or together, calcium salts and dextrose sometimes stimulated both germination and tube growth. Potassium, nitrogen, phosphorus, manganese and carbonate ions, indole-3-acetic acid, acetic acid and ammonia were ineffective although potassium as KOH or K_2CO_3 produced pronounced swelling of the intine wall.[2] Of the environmental factors the significant ones appeared to be humidity and age rather than temperature, light or acidity.

Intensive work on calcium and the related strontium and barium salts was done in Texas in the spring of 1950 and that of 1951. *Crinum* was the primary plant material. In 1953 a similar study of the effect of drying

[1] The author wishes to acknowledge Dr. Thimann's interest and kindness in suggesting pollen as plant material.

[2] In M/40–80 KOH or under mechanical pressure in weaker solutions of either KOH or K_2CO_3 swelling of the intine was followed by rupture of the extine wall and escape of the entire cell with the intine wall intact. Emerged cells were enormously enlarged with a thin wall.

was made on pollen of *Crinum*, *Nothoscordum* and *Hymenocallis*. Germination in dextrose and other sugar solutions was studied in Cambridge, Mass., June–September 1954, using pollen from a number of Irid, Amaryllid and Liliaceous genera.

Experimental technique was simple. Equipment consisted of a compound microscope with ocular micrometer, hollow-ground glass slides and covers for germinating chambers, a gold prospector's scale (accurate to 1 mg. $\pm$), C.P. chemicals, procelain-distilled water, pipettes graduated to 1/10 ml. and a 100 cc. cylinder. Pollen for each experiment was taken from a single flower or anther from which it was dusted onto single drops of the solutions. All chemicals were used in concentration series with one or more controls in water and dextrose or calcium. Stock solutions (M/5, M, 4M or saturated) were kept at 9° C$\pm$. Series were made by progressively diluting the stock one half: dilutions of one tenth were less satisfactory. To obtain progressive stages of drying, anthers from a single flower were exposed to room conditions over a period of days or weeks often with one or more held for comparison in a saturated atmosphere.

For all experiments % germination, tube length and elapsed time were recorded at the end of two or more hours. Tube growth might continue longer but most germination had occurred by the end of two hours and there was no discernible change in germination figures after four hours.

Results of the experiments may be summarized briefly.

Calcium (*at. wt. 40*). — Calcium salts were used alone and in combination with M/40 dextrose. Pollen for the 1950 tests showed no germination in water or in M/40 dextrose; 1951 material had O – 44% germination in water, O – 91% in M/40 dextrose.

All of the calcium salts tested — $Ca(NO_3)_2$, Ca Ac, $CaCO_3$, $Ca(H_2PO_4)_2$ — were effective alone in aqueous solution. They initiated germination in pollen of *Crinum* with none in water, dextrose or other solutions tested; they increased the percentage for pollen with some germination in water; they stimulated tube growth at least in the early stages.

Pollen response was dependent upon the concentration of calcium (FIGS. 1 + 2). Dilute solutions only were effective in varying degrees over a broad range (M/20 – M/40960$\pm$). Effective ranges for the different salts were: $Ca(NO_3)_2$ M/20 to M/5120–40960, Ca Ac M/20 to M/2560–40960, $CaCO_3$ sat. to M/5120, $Ca(H_2PO_4)_2$ sat. to M/2,500,000 – 10,000,000.[3] All ranges showed a well-defined optimum and often a single optimum concentration. Characteristic optima for the 1950–51 material usually included several concentrations in the M/20–M/2560 range with a single optimum between M/160 and M/640 [$Ca(H_2PO_4)_2$ sat.–M/650,000 usually with no single optimum concentration].

Optima and the dilute limit of the total range varied. They were

[3] Concentrations of the almost insoluble calcium carbonate and calcium acid phosphate were calculated from the amount of salt dissolving in 50cc. distilled water in two weeks. Since degree of solubility varies with the amount of CO_2 present and other factors, figures for these salts must be considered approximate.

characteristically at more dilute concentrations for tube length than for % germination. They shifted to more dilute concentrations also for pollen with increasing germination in water.

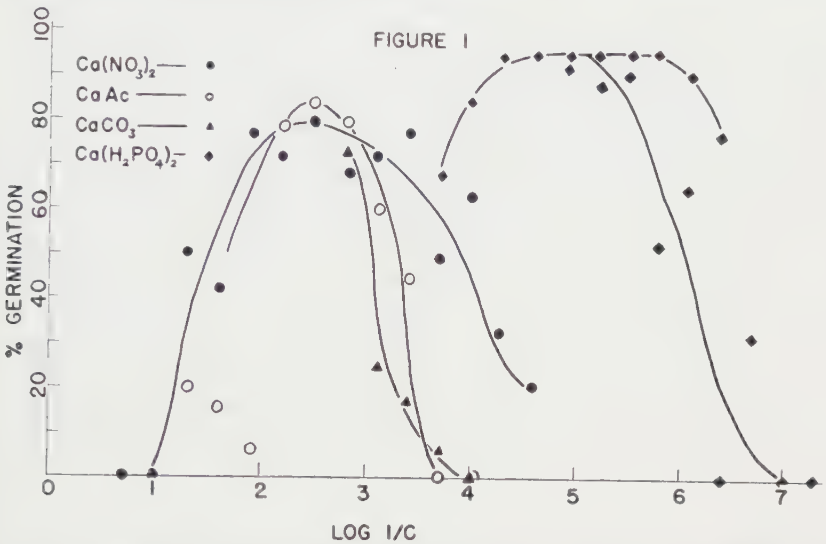

FIG. 1. Effect of calcium salts on % germination of pollen of *Crinum*; H_2O controls 0% except for the nitrate which was 18%, H_2O control.

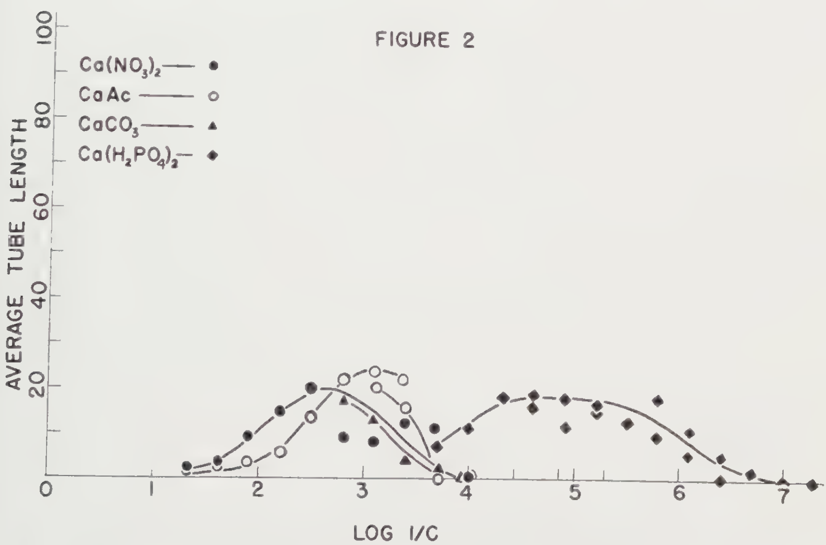

FIG. 2. Effect of calcium salts on tube length in first 2 hours of germination; water control 0. Curves for maximum tube length are similar but with values up to 50 and with a single optimum concentration for $Ca(H_2PO_4)_2$ as well as for the other three.

% germination and tube length were limited by extrusion of cell contents or by lack of response to a specific concentration of calcium. Early extrusion reduced % germination and tube length in the more concentrated portion of the range, and prevented tube formation in M/10 or stronger solutions. Lack of response seemed to be the limiting factor in more dilute solutions. Extrusion was not characteristic of this end of the range; the incidence of very short tubes declined while long tubes typical of the optimum persisted; very short tubes reappeared again in numbers only in the weakest concentrations of some experiments.

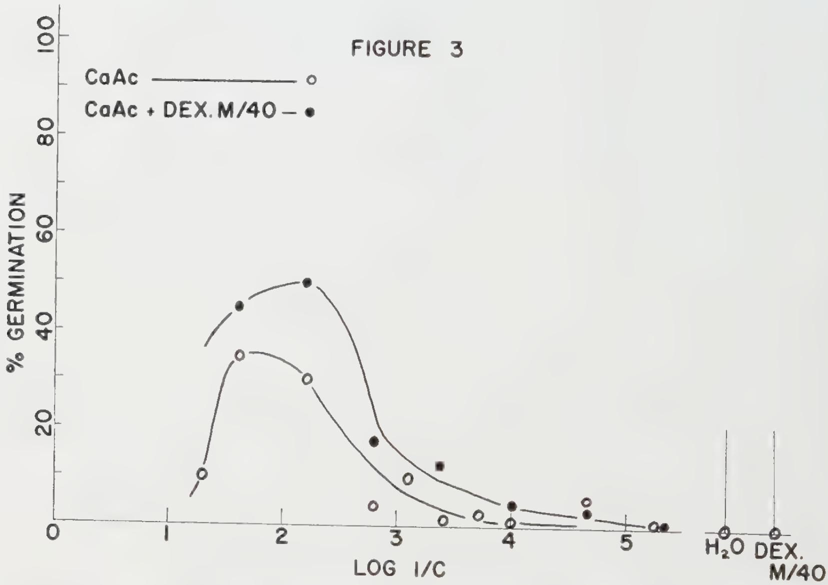

FIG. 3. Effect on % germination of adding M/40 dextrose to Ca Ac series. 1953 experiment; pollen of *Crinum*, stored 17 days.

$Ca(NO_3)_2$, Ca Ac and $CaCO_3$ were essentially alike in range, magnitude of effect and optima although $CaCO_3$ was soluble only in the more dilute portion of the range. $Ca(H_2PO_4)_2$ differed in that the total range extended to much greater dilutions while the optimum section was much broader with a recognizable single optimum concentration only in some of the data on maximum tube length.

Dextrose M/40 added to calcium solutions increased or decreased their effectiveness depending upon the condition of the pollen and the concentration of the calcium. For the 1950–51 pollen with low germination or none in water or dextrose controls, added dextrose increased % germination and tube length at most concentrations, extended the total range to greater dilutions, broadened the optimum range, eliminated the single optimum concentration or shifted it to a greater dilution, delayed or

prevented extrusion, gave more uniform tube length. The few instances of decreased germination and tube length occurred in strong or optimum concentrations and with pollen showing appreciable germination in water and/or dextrose alone.

Strontium (*at. wt. 87*). — Two salts, $Sr(NO_3)_2$ and $SrCO_3$, were tested alone and in combination with M/40 dextrose.

Both strontium salts resembled the corresponding calcium salts in their effect on pollen of *Crinum*, but were somewhat less effective. The strontium range, M/2560–5120 to M/40960+, was shorter, coinciding roughly with the dilute section of the calcium range and lacking the stronger solutions of that range. Effectiveness throughout the range declined rapidly as germination in water and/or dextrose reached zero: for increased germination and tube length, strontium was comparable with calcium only for two pollen samples with 7% germination in water and 1% in water, 15% in dextrose respectively. Tube length was often very short.

Response to added dextrose M/40 was marked, particularly where tube length and germination in strontium alone were low. It differed from that for calcium plus dextrose in that the strontium range was broadened at the concentrated as well as the dilute end and there was no shift of optima to greater dilutions. Decrease occurred only for tube length of pollen with germination in water (7%). It was appreciable and accompanied by a shift of the optimum to greater concentration.

Barium (*at. wt. 137*). — $Ba(NO_3)_2$ with and without M/40 dextrose was the single salt tested.

Pollen response to barium was even poorer than to strontium. In seven tests positive results were confined to one sample with the highest germination in water (15% vs. 0–8%). In this experiment the barium action, including the dextrose response, resembled that of the best strontium series except that the barium range was not extended by dextrose to stronger solutions.

Barium-dextrose solutions failed to produce germination in most solutions where barium alone was ineffective, but did produce germination and short tubes at low barium concentrations (M/20480+) in two barium negative samples with 6% germination in water and 0 in water, 4% in dextrose respectively.

Drying. — Material with high germination in water (50–95%) was secured for these experiments by using pollen from freshly opened anthers of the three plants selected, — *Crinum*, *Nothoscordum* and *Hymenocallis*. One characteristic of such pollen was the mixture of inhibition and stimulation that was shown in concentration series of calcium salts. Only the optimum solution or section of the range permitting germination showed any stimulation, and that often slight. All other solutions were inhibiting in various degrees (FIG. 4).

Pollen reaction to drying was marked by a decline in % germination accompanied by a change in the response to calcium and dextrose (FIG. 5).

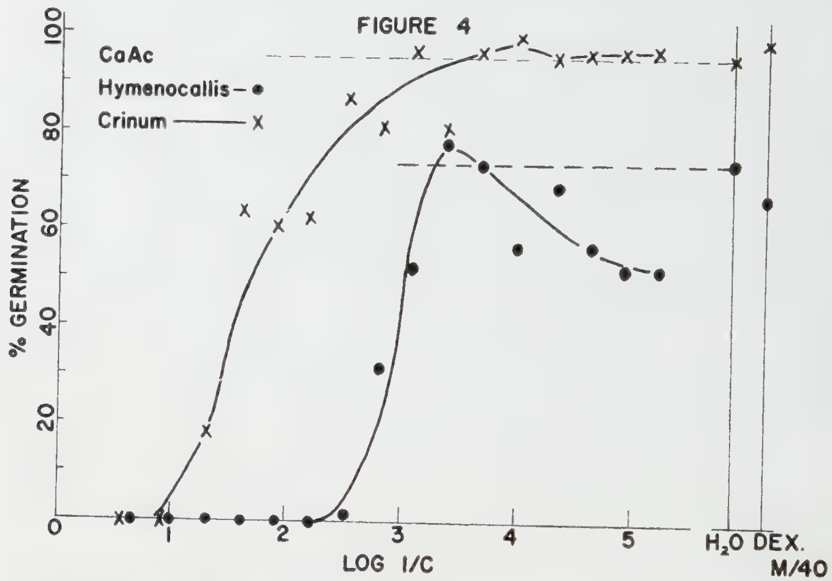

FIG. 4. Stimulation and inhibition by calcium in germination of fresh pollen with high germination in water; calcium acetate.

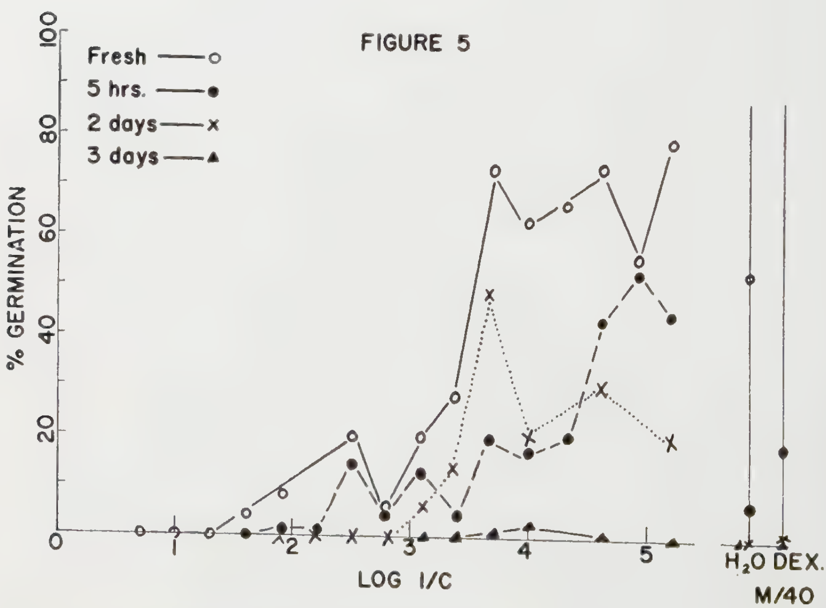

FIG. 5. Effect of drying on pollen response to Ca Ac; *Hymenocallis* pollen drying under room conditions.

These effects were counteracted or delayed by protection from drying either artificially or naturally.

Decline in germination was noted in all media. It was most rapid in water, more delayed and slower in calcium or dextrose. Water germination for pollen of *Crinum* and *Hymenocallis* commonly dropped to zero in the first 24 hours while optimum dextrose, calcium and dextrose-calcium solutions continued to show 50–90%. Germination in solutions containing calcium was retained longest: e.g. 3 days (Ca Ac) vs. 1 (H_2O) for *Hymenocallis* pollen, dry storage.

Decline in germination was retarded by storage under conditions reducing evaporation (Fig. 6). It was slower when pollen was stored in wax paper, in a moist atmosphere or at low temperatures; e.g. 5 vs. 2 day retention of water germination, moist vs. dry storage, *Crinum* pollen.

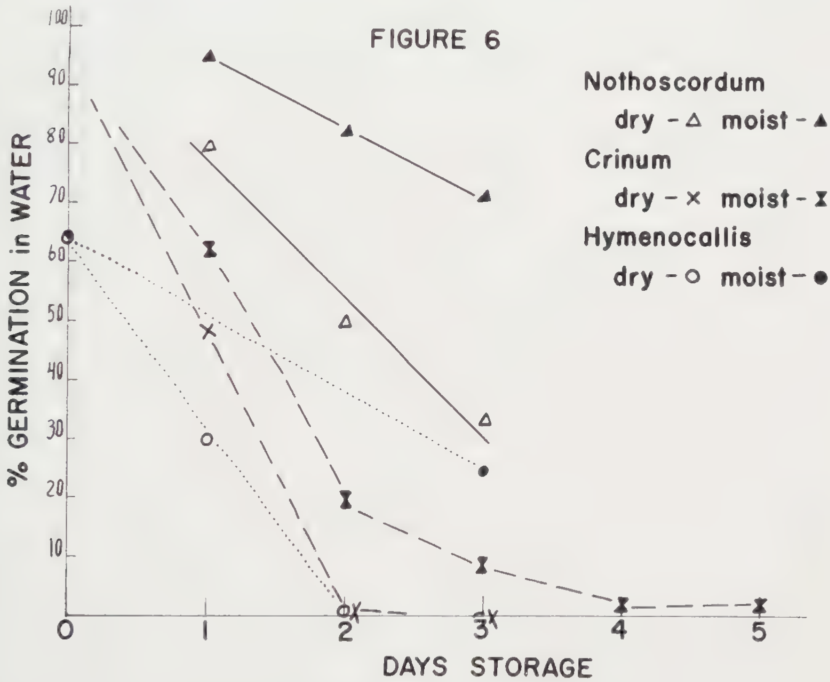

Fig. 6. % Germination in water of pollen stored in dry vs. moist air, room temperature.

Retention of germination varied with the species. With the Texas material, pollen of *Nothoscordum* showed germination longest (7 days vs. 1–2 for *Crinum* and *Hymenocallis*, water germination). Usually this species (*Nothoscordum*) did not show the sharp drop in germination in the first twenty-four hours characteristic of *Crinum* and *Hymenocallis* (water germination after one or more days often 80±% vs. 0–6% for *Hymenocallis*

and *Crinum*). In the Cambridge experiments in 1954, the two species with the longest retention of germination were *Narcissus* sp. and *Lilium regale* (up to 22 days, water germination, dry storage, and 40 days calcium-dextrose germination). Both species were characterized by very heavily massed pollen combined with copious tapetal material which, in *Narcissus*, hardened the pollen masses into cement-like pellets.

Calcium salts became increasingly effective as germinating agents in raising the % germination above that in water as pollen dried and germination in water dropped to zero (FIG. 7). At the same time an increas-

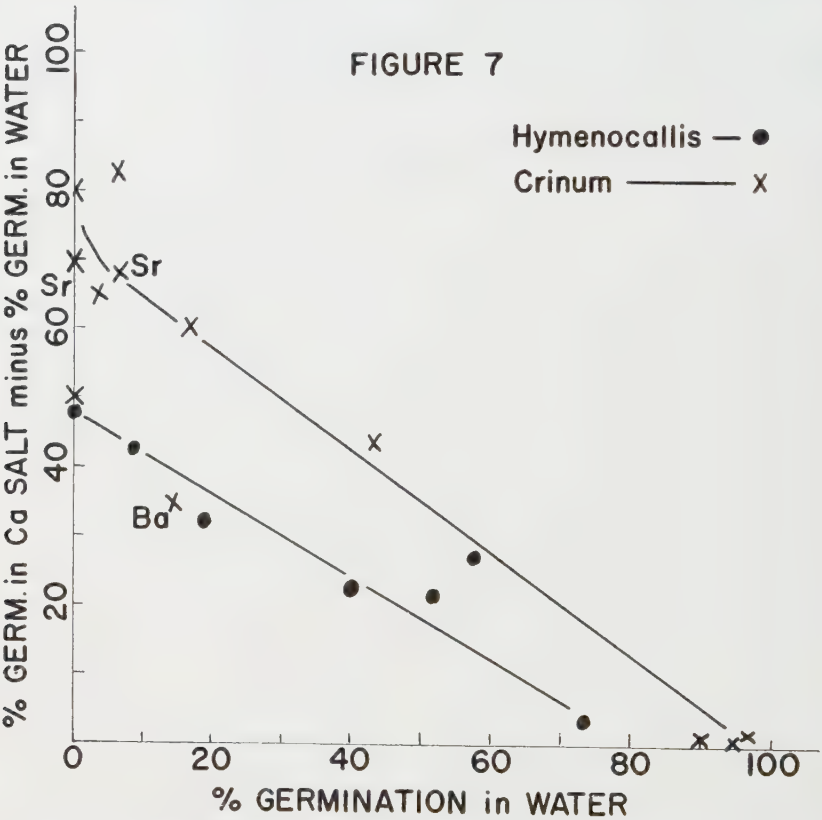

FIG. 7. Increase in effectiveness of calcium salts, Ca Ac and $Ca(NO_3)_2$, as germinating agents when pollen germination in water declines. Each figure is the optimum value for one of the longer series in 1951 and 1953 with pollen showing some germination in water or with pollen that has barely reached zero in water. A few figures for $Ba(NO_3)_2$ and $Sr(NO_3)_2$, are included.

ing number of partially or completely inhibiting concentrations became stimulating while optima and range limits shifted (FIG. 5). Optima shifted to greater concentrations as the range effective for stimulation be-

came broader until it included all concentrations permitting germination. Limits of the range in which germination occurred varied. Once established, the dilute limit shifted to stronger concentrations as drying continued. The concentrated limit was more erratic. With *Crinum* it usually remained at M/20 until the range began to shorten; with *Hymenocallis* it might shift from M/320 to M/80 first. Because of the continuous shifting the performance of a single concentration did not always follow the same course as that for the series as a whole and, particularly for originally inhibiting solutions, optimum germination might not be reached for one or more days (Fig. 8).

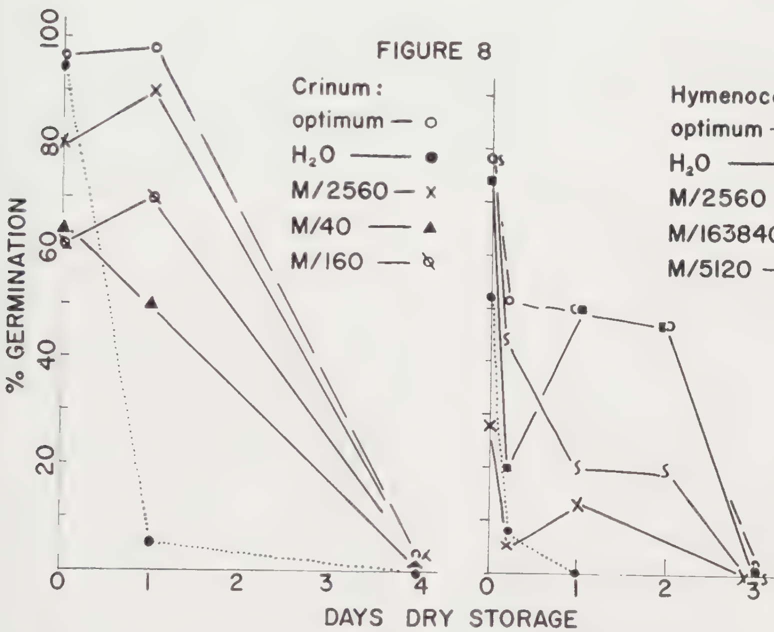

Fig. 8. % Germination of drying pollen in several concentrations of Ca Ac and in H_2O control. Pollen from one flower drying exposed to room conditions.

Observations on dextrose M/40–calcium combinations confirmed the findings of the 1950–51 experiments, but were somewhat inconclusive. To some extent the effect of added sugar was correlated with drying, but it usually seemed to be more directly dependent upon the effectiveness of the calcium. In any experiment where added dextrose M/40 increased germination in calcium solutions it was progressively more effective with concentrations showing lower percentage germination (Fig. 9); as pollen dried below $<1\%$ water germination, there was a tendency for the dextrose increase to be smaller in all but optimum concentrations. For *Crinum*, increased germination counteracted any calcium inhibition occurring in stronger solutions of the calcium range. For *Hymenocallis*, dextrose M/40

increases were confined to one series for pollen with 40% water germination and to single, weak, often barely effective dilutions (M/163840) in some other series. Added dextrose M/40 decreased germination in M/20–M/640 Ca Ac (optimum and stronger calcium concentrations) for pollen of *Crinum* with moderate germination in water (6–44%), and in M/40–M/163840± (practically the whole range) for most *Hymenocallis* pollen (0–75% water

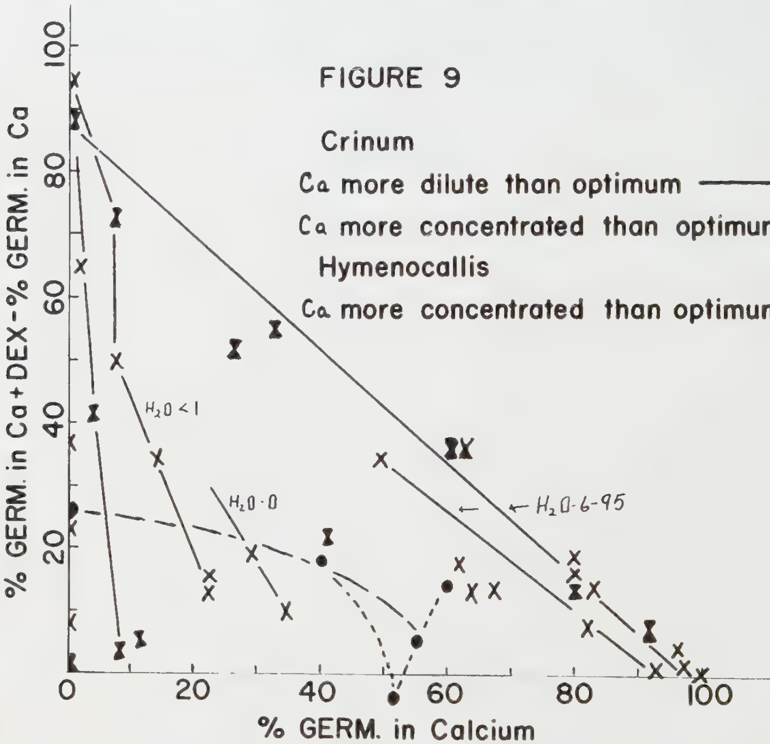

FIG. 9. Increased germination in Ca Ac with dextrose M/40 compared to % in same Ca Ac concentration alone. Lines connect data from one experiment; germination in water noted.

germination) (FIG. 10). With both plants the amount of decrease varied directly with the concentration of the calcium (FIG. 11).

Dextrose. — The most significant points shown by the dextrose series in 1954 were: (1) that dextrose like calcium was an effective germinating agent over a range of concentrations in varying degrees and subject to modification by the addition of calcium salts or by drying of the pollen (FIGS. 12 + 13); (2) that the dextrose range and effect differed from that of calcium in a number of details; (3) that dextrose under some conditions decreased or prevented germination.

The dextrose range lay mostly between M or M/2 and M/640, but it

sometimes included concentrations up to 4M (*Hymenocallis*) and dilutions to M/40960 (*Lilium regale*). Variations in range seemed to be correlated with the original condition of the pollen and the length of time that it was subsequently exposed to drying. The maximum concentration in ranges beginning at M or M/2 seemed to be fixed by pressure relationships, since pollen in stronger concentrations, with the exception of *Hymenocallis*, was unable to take up enough water to fill out the cells, and tubes in the first effective concentration were very short but intact.

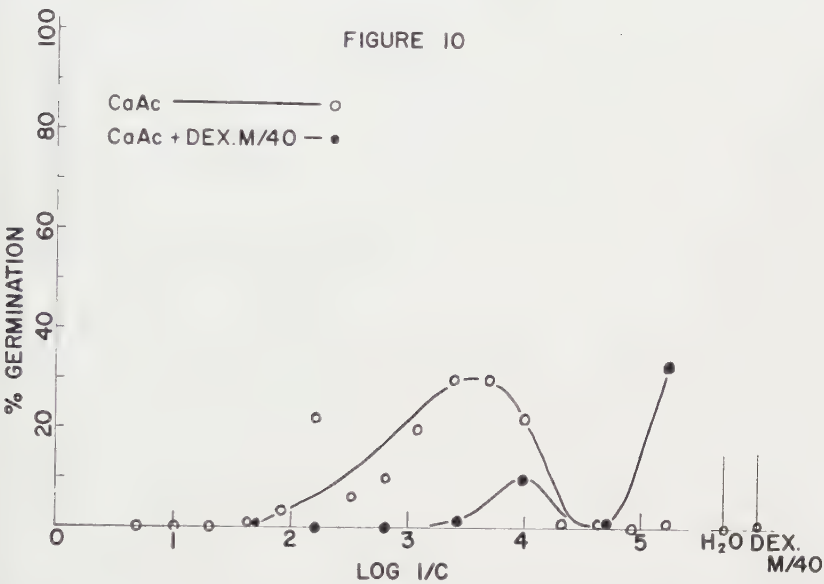

FIG. 10. Decrease in % germination of *Hymenocallis* pollen with dextrose M/40 added to calcium series.

The range for tube length at 2–4 hours was similar to but not identical with that for % germination.

The dextrose range differed from that for calcium in that it regularly included concentrations of M/10 and stronger and in that it customarily showed two well-defined optima and a very definite low. One optimum, usually the higher of the two, occurred in the more concentrated section of the range between M and M/20 (occasionally M and M/160). M/2, M/5 or M/10 were common values for this optimum. The second optimum was located in the dilute section of the range. It was more variable and occurred at a concentration between M/40 and M/320. The low which separated the two sections ordinarily was found to be M/20, M/40, M/80, occasionally M/10 or M/5 (*Crinum*). It was usually very pronounced, often an abrupt break, with germination sometimes reduced to zero or, for pollen with germination in water, to the point of partial or complete inhibition.

Drying affected germination in dextrose in some respects as it did that

in calcium solutions. As pollen dried, germination declined at a slower rate in dextrose than in water; appreciable germination continued in dextrose after it had disappeared in water; formerly inhibiting solutions of dextrose became stimulating. Optima, the low and range limits shifted. The range shortened, sometimes after an initial increase as in *Lilium longiflorum* (M/10 → M/2–M/80 → M/5–M/20), until germination was finally re-

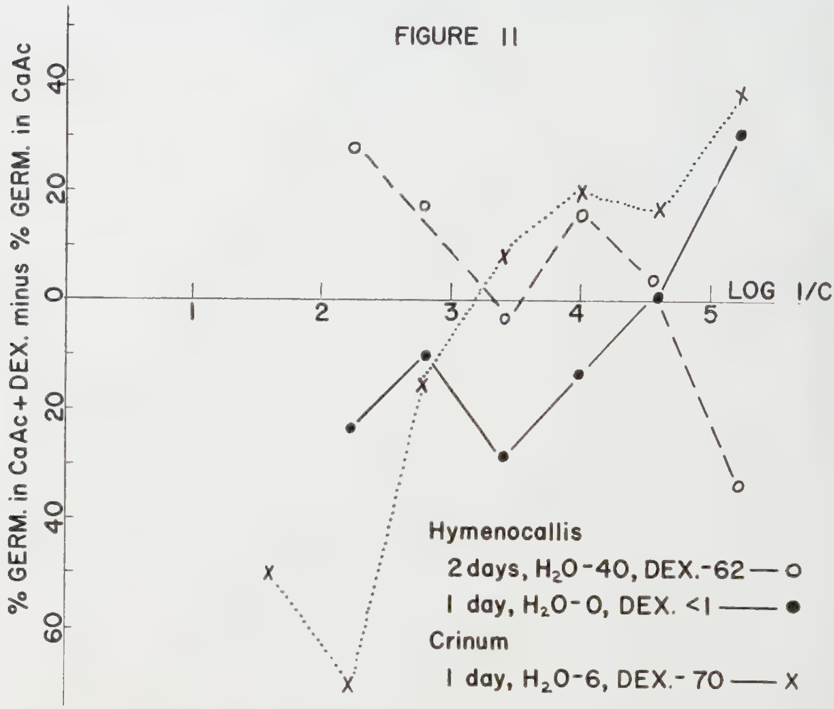

FIG. 11. Decreased germination in Ca Ac upon addition of dextrose M/40 correlated with concentration of Ca Ac.

duced often to two separated optima concentrations; germination in the stronger seemed to persist longest (*Crinum* M/10). Although both optima varied, the more concentrated one was relatively stable (*Crinum* M/2 for 4 days) while the dilute one shifted continually during drying. Selected concentrations, as in the calcium series, because of the shifting values, varied in performance so that one might show a marked increase or decline in contrast to that of the series as a whole and might reach its optimum value on the second or third day while the series as a whole continued a steady decline.

Unlike calcium, the decline in % germination in dextrose showed no apparently consistent relationship to % germination in water. It seemed to be more related to length of the drying period. Where there was suffi-

cient data on one species to be indicative, effectiveness of dextrose as a germinating agent seemed to decrease more or less irregularly with decreasing germination in water.

Calcium nitrate-dextrose mixtures showed: (1) that added calcium increased the effectiveness of dextrose series for pollen which through drying or otherwise showed lowered germination and shortened ranges in dextrose

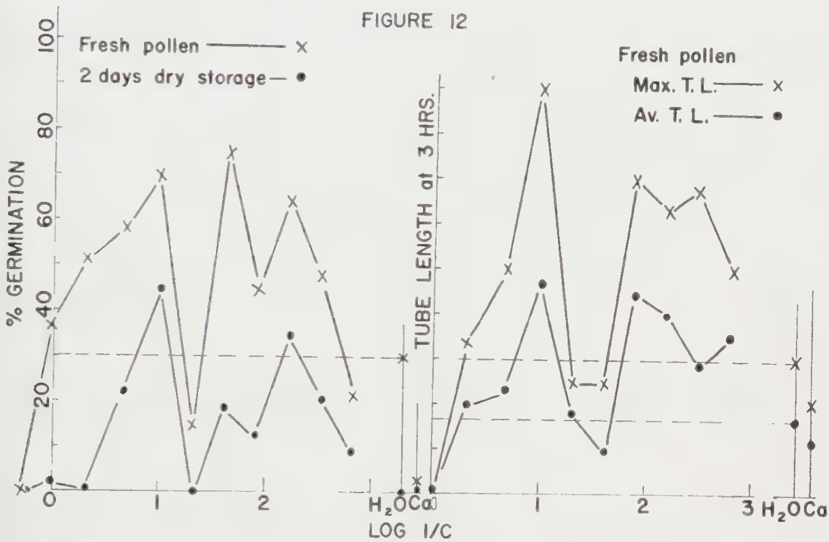

FIG. 12. Effect of dextrose on % germination and tube length of pollen of *Tulipa linifolia.*

alone; (2) that calcium concentrations ineffective in combination with dextrose M/40 were often effective combined with stronger dextrose (FIG. 13). Effectiveness varied with the concentration of calcium and of dextrose; it was modified by drying. In general a strong calcium solution (M/80) increased germination when combined with strong dextrose, decreased it when in dilute dextrose solutions; weaker calcium (M/320–M/1280) was effective throughout the dextrose range. With drying, M/80 calcium became effective in more dilute dextrose solutions while M/320, and even more M/1280, began to be ineffective.

Other Sugars. — Six other sugars have been given preliminary tests — sucrose, raffinose, levulose, galactose, arabinose and xylose. All, like dextrose but possibly in varying degrees, showed stimulation and inhibition of germination and a correlation between concentration and germination. However, pollen response in an equimolar test (M/10) including dextrose was not identical: three were positive, four negative. More tests were run on sucrose which was found to be more effective than dextrose at times; e.g. with *Iris* which did not respond to dextrose.

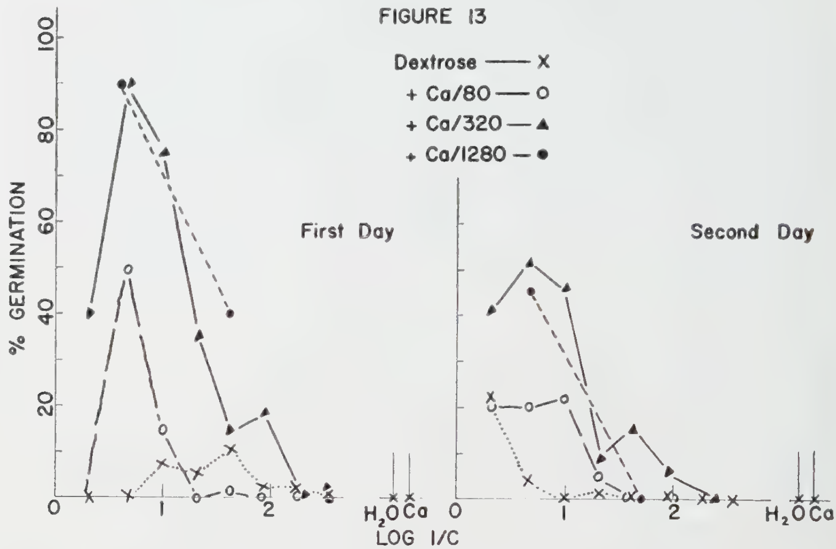

FIG. 13. Effect of Ca Ac added to dextrose series, *Hemerocallis* pollen.

DISCUSSION

It is evident from these experiments that there is at present no single technique or medium that will insure pollen germination although it may be possible to work out more or less widely applicable formulae. The 1953 experiments show clearly that drying or rate of water evaporation is an important factor in germinaiton loss which with some species and under some conditions is very rapid. The 1950–51 and 1954 tests show that this loss of germination can be compensated for by the use of calcium salts or dextrose or calcium-dextrose mixtures, but that there is no one formula for all stages of drying. Germination in water can be used as a measure of drying which may be useful in securing or determining uniformity of pollen material for experimental work or in working out a formula for use with specific material.

The data on dextrose confirm the findings of others that M/2–M/10, M/3 concentrations of dextrose may be the most generally useful sugar concentrations since germination persists longer in sugar solutions just short of being dehydrating and reaches an optimum in this part of its range. It is more difficult to select concentrations of calcium since its effect seems to be even more dependent upon the degree of drying. For use with sugar, the stronger concentrations may include more, and certainly later, stages of drying but be inferior to more dilute solutions with less dried pollen. The failure of Bungenberg de Jong to obtain favorable results with a calcium-sugar formula was undoubtedly due to the use of too strong calcium with pollen showing high germination in sugar alone (95%).

To what extent other minerals are interchangeable with calcium has not been fully determined. Strontium and barium to a limited extent are; the order of their effectiveness is inversely that of their size or atomic weight — Ca, Sr and Ba. There is nothing in these experiments to contradict the findings of Loo, Gauch and others that boron (at. wt. 10) and manganese (at. wt. 54) in combination with sugar are effective pollen germinants. A comparison between Ca^{++} and the divalent Mn^{++} would suggest that Mn^{++} which chemically resembles Ca^{++}, might fall between calcium and strontium in effectiveness and, when used alone, be more limited than calcium in use with drying pollen.

The usefulness of indole-3-acetic acid has not been thoroughly explored. There is nothing in these experiments to suggest that it may not be a useful additive to dextrose as reported by Smith and others. On the other hand, if the interpretation of the dextrose experiments is correct, its chemical structure may indicate that its effectiveness alone is limited.

More significant than any practical application is the evidence presented of factors and mechanisms involved in pollen germination.[4] Drying or rate of water evaporation is one factor operating before pollen reaches a germinating medium to reduce its ability to germinate. The presence of Ca^{++}, a mineral, and dextrose, a sugar, in the germinating medium are two other factors that can influence and control pollen germination.

That both pressure relationships and wall behavior are involved in the operation of these factors seems to be evident. Microscopic observation of pollen in strong dextrose (M/5 and stronger) indicates that stronger sugar solutions control germination through pressure relationships (osmotic, imbibitional or both) limiting the uptake of water by the pollen. The breaking of the dextrose range with the more concentrated portion showing relative stability during drying supports the conclusion that pressure control is a primary effect in this part of the dextrose range. Observation that stronger calcium concentrations (M/80), inhibiting alone or in more dilute dextrose, are effective combined with more concentrated sugar, tends to corroborate this view.

That the wall is involved in the action of calcium, dextrose and drying is indicated by practically all of the microscopic and experimental observations and is in accord with the conclusions reached by Thomas, Gauch et al for boron and its effect on pollen through cell wall and sugar transport. Stronger and inhibiting calcium solutions, intermediate inhibiting dextrose solutions limit germination by bursting apparently due to a weakened wall. Such solutions, the whole calcium range and the dilute section of the dextrose range are too weak to have any osmotic effects while neither they nor previous drying seem to affect the uptake of water by the pollen grain content. A comparison of pollen behavior in calcium and sugar solutions with that of cellulose or pectic-cellulose films indicates wall involvement; they show many points in common. The cellulose film (pectic) in pollen

[4] The author is indebted to J. Horace Faull, Jr., and Glenn A. Greathouse for their kindness in discussing some of the chemical theory involved.

is the wall. Both pollen and cellulose films take up Ca^{++} from dilute solutions; both pollen tube formation and elastic properties of a gel are affected by the sugar content; both % germination of pollen and water uptake of cellulose are decreased by previous drying.

That the intine rather than the extine wall is involved can be established by direct observation. The latter remains inert while the intine wall initiates tube formation and forms the wall of the developing tube.

A consideration of the known behavior of cellulose films and of the theoretical chemistry of high polymers (Meyer; Mark and Tobolsky) leads to the conclusion not only that the wall is involved, but that the calcium, dextrose and even drying act directly on the intermolecular structure which affects the properties of the wall and in particular its plasticity.

Hydration and dehydration are apparently of primary importance in determining the plasticity of cellulose for which water is one of the leading plasticizers. That water, aside from its rôle of creating pressure from within the pollen cell, plays an important part in germination is evident in the experiments on drying of pollen. In its general relation to rate of water evaporation, loss of germination through dehydration parallels the expected loss of plasticity in a cellulose film.

The rôle of calcium may be that of a hydrogen bond opener which breaks the H-bonding between linear cellulose groups to promote water uptake and plasticity. By forming calcium ionic bridges between cellulose members it may also increase the toughness of the wall. That both of these effects are involved seems likely. Tube formation depends upon both the plasticity of the wall and sufficient toughness to withstand internal pressure incident to its formation, although lack of toughness can be compensated for at least in part by reduction or counteraction of the pressure. Cf. pollen behavior in stronger concentrations of the dextrose range. Evidence supporting this view is found in the correlation between % germination of pollen and the concentration of Ca^{++} (calcium content is a determinant in the development of an elastic gel), and in the modification of the calcium concentration vs. % germination curve by drying of pollen. Since two properties are needed in proper balance, both directly dependent upon the Ca^{++} but one also involving subsequent hydration, they might be expected to develop at somewhat different rates with increasing Ca^{++} concentration. This would account for the irregularities in the Log 1/C vs. % germ. curves beyond those due to experimental error. Excessive fluidity or stiffness, both inherent in the effects of increasing Ca^{++} concentration would limit the usefulness of the wall for tube formation and make it liable to the breaking under pressure characteristic of stronger calcium solutions.

The order of effectiveness of Sr^{++} and Ba^{++} and their greater restriction to pollen with specific hydration of the wall, if one may take % germination in water as a measure, is what would be expected from their size or atomic weights.

The apparent deviation of $Ca(H_2PO_4)_2$ in range limits and optima is not necessarily contradictory to the evidence in the similarity of the other three salts that calcium is the effective ion. The rôle of phosphorus in

sugar metabolism has long been recognized and it is known to be a degradent for cellulose. The phosphate ion might very well produce sugar within the cellulose wall which would have an effect on pollen similar to the addition of dextrose to the medium. Inspection of the $Ca(H_2PO_4)_2$ concentration vs. % germination curves and those of calcium-dextrose M/40 mixtures for comparable material shows a similar broadening of the optimum range, tendency to eliminate a single optimum concentration, extension of total range to greater dilutions and % germination increase beyond that of the Ca^{++} effect as defined by the other three salts alone for similar pollen.

The rôle of sugar beyond that of pressure control may be similar to that of calcium in increasing hydration and plasticity and in strengthening the wall. Inasmuch as interposition of the sugar molecule is through hydrogen bonding and not ionic bridges, its strengthening effect would be less than that of calcium. Because of its larger size, ability of the sugar molecule to penetrate drying cellulose is less even though its linear character may give better penetration than its molecular weight would indicate. The linear sugar structure may also provide additional slippage not found in the Ca^{++}, while its bulk opens more space for water.

Evidence is much the same as for calcium and emphasized by the similarities between the calcium and dextrose effects. The differences to be expected because of the somewhat different physical-chemistry of the sugar molecule and the Ca^{++} are to be found in the shortening of the dextrose range at the dilute end, in the differences in range detail such as optima and irregularities in this same section, and in the somewhat poorer performance of dextrose with drying pollen.

The outstanding difference between the calcium and dextrose ranges is the inclusion of concentrations stronger than M/20 and the break separating the range into a dilute and a strong section. Both may be attributed to the pressure effects of dextrose which seem to dominate the stronger section while evidence of wall conditioning is observable in the dilute section. The break occurs at intermediate concentrations where pressure control is declining and wall condition poor. Variation in location and magnitude of the break is a part of the variation in the dilute section of the range already discussed.

Dextrose inhibition like that of calcium, it may be worth noting, is easily explainable in terms of wall conditioning.

In calcium-dextrose mixtures the apparent dominance of the calcium, particularly with drier pollen, may be attributable to its better penetration of the cellulose lattice which might facilitate entry of the dextrose molecule. Otherwise the sometimes favorable, sometimes unfavorable response to the mixtures is what might be expected from the large number of not always compatible wall effects involved.

Tube length in the first hour or so, to the extent that it is a measure of germination, may be explainable in the same terms as pollen germination. But the problem of later development is a separate one already beginning

to appear in the early tubes as an inspection of the data on tube length shows.

In conclusion it may be noted that the universal presence of sugar and calcium in plants and the equally universal problem of drying make the possible application of the findings of this study to ecology, floral structure and plant distribution most intriguing.

SUMMARY

Experiments to show the effects on pollen germination of Ca^{++}, Sr^{++}, Ba^{++}, dextrose, other sugars and drying have been reviewed and discussed both with respect to any practical application of the findings and to the evidence they present of factors and mechanisms involved in pollen germination.

A possible mechanism in terms of wall conditioning through the control of hydration and plasticity by drying, Ca^{++} (mineral) and dextrose (sugar) is suggested.

CAMBRIDGE, MASS.

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COMPARATIVE MORPHOLOGY OF THE FOLIAR SCLEREIDS IN BORONELLA BAILL.

ADRIANCE S. FOSTER

With five plates

INTRODUCTION

THIS ARTICLE REPRESENTS the continuation of the writer's studies on the comparative morphology and ontogeny of foliar sclereids in the dicotyledons. It is now evident that from a topographical point of view, laminar sclereids may be classified into two main types, viz.: diffuse and terminal. The diffuse pattern is illustrated by *Trochodendron* (8), *Drimys*, *Belliolum*, *Bubbia* and *Zygogynum* (3), *Illicium* (4) and *Olea* (1), in which the sclereids occur as solitary or clustered idioblasts distributed in the mesophyll without consistent relation to the veinlets or veinlet-ends. In contrast, the sclereids of certain other genera, such as *Mouriria* (10, 18), *Coryphadenia* (19) and *Memecylon* (10, 25) are predominantly terminal, i.e., they lie in direct contact with the tracheary elements of the veinlet endings. Aside from their diagnostic value in systematic anatomy (1, 10, 18, 19, 21) these two patterns of sclereid distribution pose a problem of considerable morphogenetic interest. Ontogenetic studies have demonstrated that the terminal sclereids of certain species of *Mouriria* and *Memecylon* originate from initials which are located from the beginning at the terminations of the procambial network of the developing lamina (11, 20). Diffuse sclereids on the contrary arise from cells in the future palisade or spongy parenchyma — or from cells in both regions — and in general are unpredictable in their distribution and time of origin in the developing lamina (9, 12). Experimental studies on the physiological factors which condition the expression of the diffuse and terminal patterns of sclerenchymatous idioblasts are highly desirable but as far as the writer is aware, have never been attempted.

In an effort to extend our limited knowledge of the morphology and diagnostic value of terminal sclereids, the genus *Boronella* was selected for investigation. This genus of the Rutaceae consists of three or possibly four species of low shrubs endemic to New Caledonia (7, 16). The selection of *Boronella* was prompted by the discovery by Schulze (24) that in several species of *Boronia*, a genus presumably closely-allied to *Boronella* (5, 7), the leaves are characterized by branched sclerenchymatous idioblasts which almost invariably lie at the terminations of the veinlets. The writer not only confirmed the accuracy of Schulze's observations on *Boronia* but found that terminal sclereids are also a prominent aspect of leaf structure in *Boronella*. The present study, seriously handicaped by the lack of fresh or preserved leaf material, represents apparently the first anatomical

investigation which has been made on *Boronella*. It is hoped that it may shed some light on the structure and systematic relationships of an interesting New Caledonian endemic.

SYSTEMATICS OF BORONELLA

Baillon (5) based his original description of the genus *Boronella* upon the species *B. pancheri*. Since the time of Baillon, the following additional species have been proposed: *B. francii* Schltr. (23), *B. verticillata* Guill. ex Engler (14), *B. parvifolia* Baker f. (6) and *B. crassifolia* Guill. (15). In a paper devoted to the problems of synonomy which have arisen in the systematic treatment of *Boronella,* Guillaumin (14) concluded that *B. pancheri* Baill. and *B. francii* Schltr. are identical and that they are not distinguishable from Baker's *B. parvifolia*. Consequently, as presently conceived by Guillaumin (16), the genus now consists of three species, viz.: *B. pancheri*, *B. verticillata* and *B. crassifolia*. Leaf material of each of these species as well as samples of several collections of *B. francii* have been examined in the present investigation.

MATERIAL EXAMINED

The writer desires to record his thanks to the following individuals who contributed the herbarium material of *Boronella* used in the present study: Dr. Richard A. Howard, Director of the Arnold Arboretum and Dr. Clarence E. Kobuski, Curator of the Arnold Arboretum and the Gray Herbarium of Harvard University (AA and GH); Dr. A. C. Smith, of the U. S. National Herbarium (US); Dr. H. L. Mason, of the University of California (UC) and Dr. Jacques Leandri, of the Muséum National d'Histoire Naturelle of Paris (Paris). Special appreciation is also expressed to Dr. G. F. Papenfuss for his aid in forwarding the herbarium material from Paris and to Dr. Knowles A. Ryerson who sent the writer dried leaf specimens of *B. pancheri* which had been collected by H. S. McKee at Marais Kiki, Plaine des Lacs, New Caledonia (*McKee 1107*).

A complete list of the sources of the leaf specimens used in this study is as follows:

B. CRASSIFOLIA Guillaumin. *Buchholz 993a* (UC, US, AA); *Franc 2439* (AA); *Balansa,* Feb. 1872 (Paris).

B. FRANCII Schltr. *No. 247 "F.P."* (AA); *Franc 247A* (AA); *Franc 247* (AA); *Franc 247* (US).

B. PANCHERI Baill. *Bonati 247* (UC); *Franc* Jan. 5, 1916 (US); *Prony s.n.* (AA); *Le Rab,* 1900–1910 (Paris); *Franc 247* (GH).

B. VERTICILLATA Guill. ex Engler. *Franc 1618* (AA); *Franc 1618P* (AA); *Franc 1618,* sér. A (UC); *Franc 3018* (UC); *Franc 1618* (GH); *Balansa 1061* (US); *Bernier,* Oct. 1900 (Paris).

TECHNIQUE

In order to secure satisfactory preparations of cleared laminae, it proved essential to modify the general procedure used by the writer in his studies of leaf venation (13). Even after a prolonged sojourn in dilute NaOH, the leaves of all species of *Boronella* studied were opaque and dark in color. The problem was solved by transferring the partially cleared leaves — after very thorough washing in distilled water — to a strong aqueous solution of chloral hydrate (250 gr. chloral hydrate in 100 cc. H_2O). Within a few days, the leaves became semi-transparent and were then ready to be washed in water, dehydrated in alcohol and stained in safranin.

The technique used in processing the herbarium leaf specimens so that useful serial paraffin sections could be obtained was devised by Dr. Francis V. Ranzoni whose assistance and skill is gratefully acknowledged. His method is as follows. The material is first aspirated in 70% alcohol, washed thoroughly for about an hour in hot tap water and then treated briefly (for about 15 min.) in 5% NaOH. The next step consists in placing the specimens in "Stockwell's Solution" (17, p. 85) for 48 hours or longer, which results in making the material lighter in color and somewhat transparent. Then, in order to insure smooth sectioning, the leaf material is placed in a 16% solution of hydrofluoric acid for 7–14 days; this treatment not only softens the tissues but results in complete elimination of discoloration. After treatment with acid, the material is very thoroughly washed in water and then brought into pure paraffin by the usual tertiary-butyl-alcohol technique. Serial transections of the laminae were cut at 10–12 microns in thickness and stained for about 24 hours in a 1% solution of safranin in 50% alcohol. The use of tannic acid-iron chloride, in conjunction with the safranin, yielded unsatisfactory preparations characterized by the overstaining of all lignified elements, in particular, the sclereids.

As in previous investigations, the writer is greatly indebted to Mr. Victor Duran for his skill in photographing the preparations used as illustrations in this paper.

GENERAL HISTOLOGY OF THE LAMINA

Before describing in detail the form and distribution of the foliar sclereids, it will be essential to outline the salient features of the histology of the lamina of *B. crassifolia, B. pancheri* and *B. verticillata* as revealed by a study of clearings and serial sections. It must be emphasized that the observations recorded below are based on a study of samples of a very few collections of leaves of these species and hence may not adequately depict the range of inter- and intraspecific variations which exist.

1. **Epidermis.** In all three species, the outer walls of the cells of the upper epidermis are very conspicuously thickened and, as shown in Figs. 8–10, are more or less separated from one another along their outermost radial points of normal contact. Sections of one collection of *B.*

pancheri (*Prony s.n.*, AA), however, showed a better preservation of the epidermal layer and a clearly stratified character of the thickened portions of the outer tangential walls. Stomata are restricted to the lower epidermis and as shown by FIG. 10, the guard cells are apparently extremely small in transectional view.

2. **Hypodermis.** A distinctive aspect of laminar histology is the presence of a well-defined hypodermis situated directly beneath the upper epidermis. In the two collections of *B. pancheri* examined and in *B. verticillata* (*Franc 1618* UC), the hypodermis consistently is a single layer of tightly-joined cells (FIGS. 9 and 10) while in *B. crassifolia* (*Buchholz 993a* UC) the hypodermis consists of two (in certain areas apparently three) layers of cells separated from one another by small intercellular spaces (FIG. 8). It seems entirely possible that the two-layered character of the hypodermis in *B. crassifolia* may prove a reliable diagnostic character of this species. The true morphological nature of the foliar hypodermis of *Boronella*, i.e., whether a derivative of the protoderm and hence the inner part of a true multiple epidermis, or a tissue independently derived from the ground meristem, can only be solved by ontogenetic study of adequately fixed material. (Cf. Foster (12, p. 70–71) for a discussion of the concepts of hypodermis and multiple epidermis.)

3. **Mesophyll.** As might be anticipated, this delicate region of the lamina is more or less distorted in sections of herbarium leaves. Its general structure however is evident and a well-defined palisade tissue, varying in thickness in the three species, is rather sharply demarcated from the spongy parenchyma (FIGS. 8–10).

4. **Secretory Cavities.** The secretory cavities or internal "glands," so characteristic of leaves in the Rutaceae (7, 24), are well-developed in *Boronella* although interesting differences appear to exist between the three species with reference to the position of the cavities in the mesophyll. In both collections of *B. pancheri* examined, the palisade parenchyma is devoid of secretory cavities and these structures are only found scattered in the spongy parenchyma directly below the lower epidermis of the lamina. Secretory cavities in *B. crassifolia* and *B. verticillata*, in contrast, develop hypodermally in both the palisade and spongy parenchyma and appear to be relatively abundant (FIG. 8). Whether these differences are consistent however can only be fully demonstrated by a more comprehensive survey of a wide range of material.

5. **Venation patterns and vein structure.** As far as could be determined, the simple, emarginate, petiolate leaves of *Boronella* are characterized by their opposite or whorled phyllotaxis and by a unilacunar type of node. The small obovate or spatulate leaves of *B. pancheri* differ in the pattern of major venation from the much larger leaves of the other species because of the extension into the lamina, as branches of the single petiolar bundle, of a pair of strong lateral veins. This type of "palmate" venation contrasts with the more strictly pinnate arrangement of the main lateral veins in *B. crassifolia* and *B. verticillata*. In all three species, however, the major and minor veins are extensively anastomosed and form a con-

spicuous network with very numerous veinlet endings which commonly lie in direct contact with solitary or clustered branched sclereids (FIGS. 1, 2, 3, 4, 7).

Transections of the lamina reveal that the bundles are collateral in structure, and that the midrib bundle and the major lateral veins are accompanied by more or less well-developed strands of fibers (FIGS. 8–9). In *B. pancheri*, the midrib bundle is completely ensheathed by fibers while in the other species the fibers constitute two separate strands of tissue, one located at the periphery of the phloem and the other adjacent to the protoxylem of the bundle. The small veins and veinlet ends, in contrast, are devoid of fibers.

DISTRIBUTION OF SCLEREIDS IN THE LAMINA

An intensive study of cleared laminae from the various collections of *Boronella* has revealed two rather distinct trends in the topography of the branched sclereids. In all leaf specimens of *B. crassifolia* and *B. verticillata* and in certain collections of *B pancheri*, the sclereids are predominantly terminal in position at the veinlet endings and sclereids isolated from the vascular system are comparatively rare in occurrence. As seen at low magnification, the consistent development of sclereids at virtually all vein endings throughout the lamina of *B. crassifolia* presents a striking and definitive pattern (FIG. 1). An entirely similar condition is characteristic of the leaves of *B. verticillata* and in both of these species, veinlet endings devoid of one or more ramified sclereids, are extremely infrequent. At higher magnification, the form and abundance of sclereids associated with the veinlet terminations is plainly revealed. In both *B. crassifolia* and *B. verticillata* there is a conspicuous tendency for groups of 2–3 sclereids to develop at the majority of the veinlet endings although in many instances solitary sclereids occur (FIGS. 2, 3, 7). Because of the complex branching of the sclereids, it is often impossible to determine accurately the exact number of sclereids in a given terminal group, as is clearly demonstrated by FIGS. 2 and 3.

The direct contact between solitary or clustered sclereids and the terminal tracheary elements of the veinlet can usually be readily seen in cleared leaves but is very convincingly demonstrated in transactions of the lamina. FIGURE 8, for example, shows at relatively low magnification a large vertically oriented sclereid at the terminus of a short veinlet in the leaf of *B. crassifolia*. The intimate "ball and socket" relationship between a pair of large ramified sclereids and the tracheary cells of the veinlet end is exceptionally well-displayed in the transection of the lamina of *B. verticillata* shown in FIG. 9. Because of the intricate pattern of the minor venation and the recurved orientation of a large proportion of the veinlet endings, transections comparable to those shown in FIGS. 8 and 9 are infrequent but sufficient examples were studied to demonstrate the precise nature of the contact between sclereid and tracheid.

In striking contrast to the predominance of terminal sclereids in *B.*

crassifolia and *B. verticillata*, the topography of the foliar sclereids in *B. pancheri* and *B. francii* is highly variable. Remarkable fluctuations occur with reference to sclereid position, not only between leaves from different collections but even between the leaves of the same herbarium specimen. On the whole, the general trend is towards the development of diffuse sclereids, which occur as isolated or clustered idioblasts unrelated to the veinlets or veinlet ends (FIGS. 5 and 10). This is the condition in the leaves of the majority of the collections of *B. pancheri* and *B. francii* which were examined, although in nearly all cleared preparations it was possible to find occasional sclereids lying at the terminations of some of the veinlets. Two of the most interesting examples of topographic fluctuations are, however, particularly worthy of emphasis in this connection. A survey of ten leaves from one of the collections of *B. francii* (*Franc 247* US) revealed comparatively few diffusely-arranged sclereids and the majority of them were strictly terminal in position. In size and form, the leaves of this specimen entirely agree with those of other collections of *B. pancheri* and *B. francii*. The most remarkable example of fluctuation encountered in *B. pancheri* was provided by the collection *Bonati 247* (UC). In two leaves, the great majority of the sclereids were situated at the veinlet ends (FIG. 4) while in many other leaves of this same specimen, most of the sclereids are scattered in the mesophyll distal to the veinlets (FIGS. 5 and 10).

The conspicuous trend towards the development of diffuse sclereids in *B. pancheri* and *B. francii* seems to be correlated with the dilation of one or more of the terminal tracheids of the numerous "free" veinlet endings. In some collections, only occasional veinlets terminate in enlarged or lobed tracheid-like cells (FIG. 5). But in several of the other collections, especially *B. pancheri*, *Franc* Jan. 5, 1916 (US), most of the veinlet ends are more or less dilated or club-shaped, and consist of one or of many ovoid or irregular tracheids. As shown in FIG. 6, the diffuse sclereids may be sparsely distributed between the veinlets, but in other collections with dilated vein endings (e.g., *B. francii No. 247 "F.P.,"* AA; *B. pancheri Le Rab*, 1900–1910, Paris), the sclereids are excessively abundant and closely packed within the mesophyll.

FORM AND STRUCTURE OF THE SCLEREIDS

The predominant type of foliar sclereid in *Boronella*, regardless of its topography, is a profusely ramified cell, the branches radiating in various planes from the main or central "body" (FIGS. 1–7). As seen in transections of the lamina, it is evident that the vertically-directed branches of the sclereid extend into both the palisade and spongy parenchyma tissue (FIGS. 8–10). No examples however were seen in which the tips of any of the branches were in contact with either layer of the epidermis. In general, the sclereids conform to the type of so-called "astrosclereids" of Tschirch (26) although in *B. crassifolia* (*Buchholz 993a*, all collections), some of the sclereids are decidedly elongate and fiber-like in form. As shown in

FIG. 3, such fusiform sclereids commonly lie at one side of a veinlet and, when situated marginally, are branched in a bizarre and distinctive manner.

The thickness of the secondary wall of the sclereids tends to fluctuate considerably. In all collections of *B. crassifolia* examined, the lumen is reduced to a narrow channel and pits are sparse and vestigial in character (FIGS. 3 and 8). The sclereids of *B. verticillata* and *B. pancheri*, on the contrary, exhibit a more conspicuous lumen and small scattered pits are evident under high magnification at various points in the "body" of the cell (FIGS. 9 and 10). The nature of the pitting at the point of contact between a terminal sclereid and the adjacent tracheid however could not be satisfactorily determined from transections of herbarium leaves.

In addition to the branched type of terminal or diffuse sclereid, small brachysclereids or "stone cells" were observed in some of the leaves of collections of *B. pancheri* and *B. francii*. These idioblasts are never abundant and in all cases are restricted to one or two widely isolated areas in the lamina. Commonly the brachysclereids occur in small dense clusters and may in addition accompany, for short distances, the slender veinlets. There is no evidence that such cells were produced in response to injury and their sporadic occurrence and limited distribution within the species of *Boronella* is indeed puzzling.

The only other type of specialized idioblast occasionally encountered in *B. pancheri* and *B. francii* was a small tracheid-like cell resembling in its form and helical thickenings the modified tracheids of the vein endings. Idioblasts of this character were not seen in the leaves of either *B. crassifolia* or *B. verticillata*.

SUMMARY AND DISCUSSION

One of the most interesting results of this study is the discovery of the remarkable degree of fluctuation in the distribution of strictly terminal sclereids in the leaves of *Boronella*. Two of the species, *B. crassifolia* and *B. verticillata*, closely agree in having the majority of the foliar sclereids at the veinlet endings (FIGS. 1, 2, 3, 7, 8, 9). In marked contrast, sclereid topography is extremely unstable in the leaves of *B. pancheri* and *B. francii*. The range in variation in these plants includes the predominantly diffuse pattern of sclereids characteristic of most of the collections (FIGS. 5 and 6) and the combination of both terminal and diffuse branched sclereids which occurs within the different leaves of the same collection (FIGS. 4–5). The marked trend towards the development of modified, club-shaped free veinlet ends (FIGS. 5 and 6) and the sporadic occurrence of small brachysclereids, represent additional aspects of foliar specialization in *B pancheri* and *B. francii* which were not encountered in the two other species.

The systematic implications of the variations in sclereid position in *Boronella* represent problems which demand for their solution the comparison of a much wider range of material than has been possible in the present investigation. Future studies should include a careful examination of the flower because the writer has observed a profuse development of *both*

terminal and diffuse ramified sclereids as well as "stone cells" in the sepals and petals of two collections of *B. pancheri.* The structure and topography of sclereids in the flowers of the other species possibly might provide additional information of morphological and systematic value as Morley (18, 19) found in his studies of floral sclereids in *Mouriria* and *Coryphadenia.* The nature of the interspecific relationships between the entities which have been included in the present study however can only be clarified by comprehensive anatomical and ontogenetic studies on all parts of the plant. But at least from the standpoint of the structure and topography of the foliar sclereids, there appears to be no valid distinction between *B. pancheri* Baill. and *B. francii* Schltr. In accordance with the conclusions of Guillaumin (14) and Engler (7), *B. francii* should probably be regarded as conspecific with *B. pancheri.*

In conclusion, it must be emphasized that the systematic distribution of terminal foliar sclereids in the dicotyledons proves to be considerably more widespread than has been realized. Sclereids which are predominantly situated at the veinlet endings in the lamina occur in the Melastomaceae (10, 18, 19, 25) Hamamelidaceae (11, p. 511), Polygalaceae (11, p. 511), Capparidaceae (21, 22) and Rutaceae (24). Very recently the writer has discovered that the large profusely branched sclereids in the leaflets of *Hannoa klaineana* Pierre & Engler (Simaroubaceae) are predominantly terminal in position. Further morphological explorations combined with intensive ontogenetic study are clearly needed and the results may be expected to yield information of systematic and phylogenetic importance.

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EXPLANATION OF PLATES

PLATE I

Fig. 1. *Boronella crassifolia. Buchholz 993a.* Portion of cleared lamina showing terminal sclereids. × 17. Fig. 2, the same, showing solitary and clustered terminal sclereids at all veinlet endings. × 85.

PLATE II

Fig. 3. *Boronella crassifolia. Buchholz 993a.* Portion of marginal area of cleared lamina showing clustered terminal sclereids. Note large branched fusiform sclereids at top and near center of photomicrograph. × 85. Fig. 4.

Boronella pancheri. Bonati 247. Portion of cleared leaf showing well-defined terminal sclereids. × 85.

PLATE III

FIG. 5. *Boronella pancheri. Bonati 247.* Portion of cleared leaf from same collection represented in FIG. 4. Note prevailingly diffuse sclereids and the enlarged irregular tracheids of certain veinlet ends. × 85. FIG. 6. *Boronella pancheri. Franc*, Jan. 6, 1916. Portion of cleared leaf showing modified and dilated veinlet endings. Note diffuse but sparsely-distributed sclereids. × 85.

PLATE IV

FIG. 7. *Boronella verticillata. Balansa 1061.* Portion of cleared leaf illustrating solitary and clustered terminal sclereids. × 85. FIG. 8. *Boronella crassifolia. Buchholz 993a.* Transection of lamina showing at the right of the large collateral bundle a branched sclereid in contact with a veinlet ending. Note also the two-layered adaxial hypodermis and the conspicuous "gland" beneath lower epidermis at left. × 124.

PLATE V

FIG. 9. *Boronella verticillata. Franc 3018.* Transection of lamina showing contact between a pair of ramified sclereids and the tracheary elements of a short veinlet ending. Note single-layered adaxial hypodermis which is separated from epidermis as a result of processing the leaf for sectioning. × 218. FIG. 10. *Boronella pancheri. Bonati 247.* Transection of lamina showing isolated branched sclereid lying free in mesophyll. Note single-layered adaxial hypodermis and the small size of the guard cells of the stomata in the lower epidermis. × 173.

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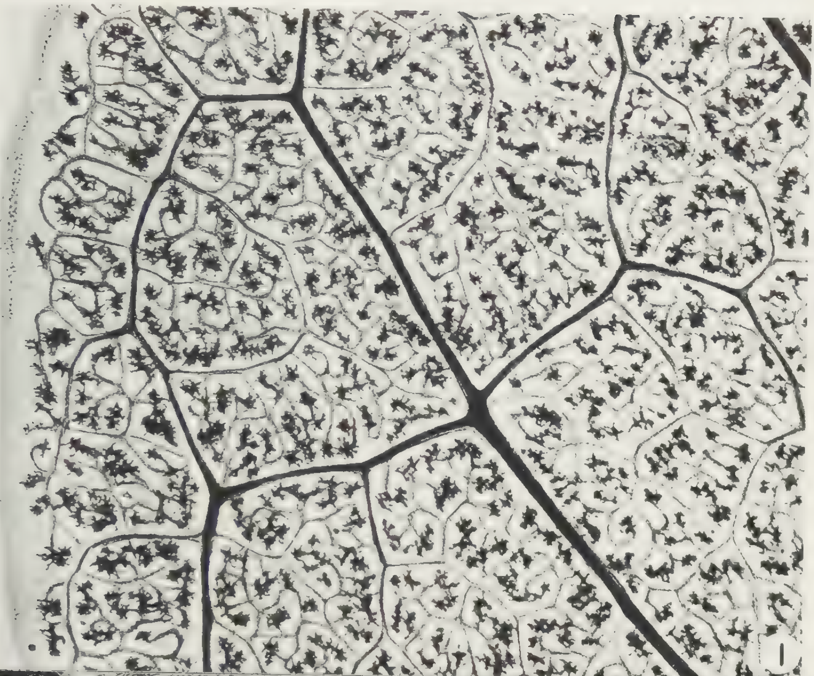
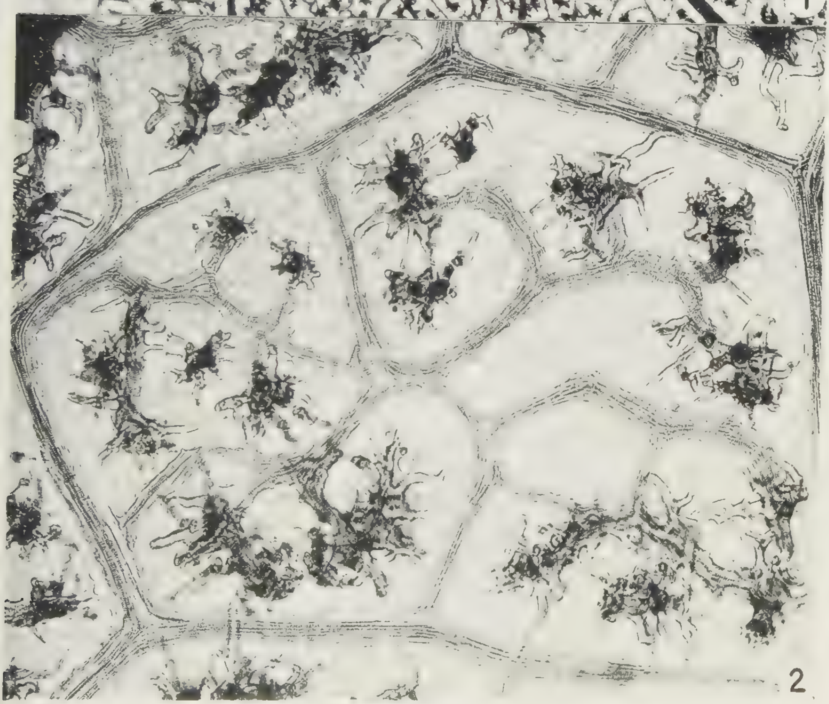
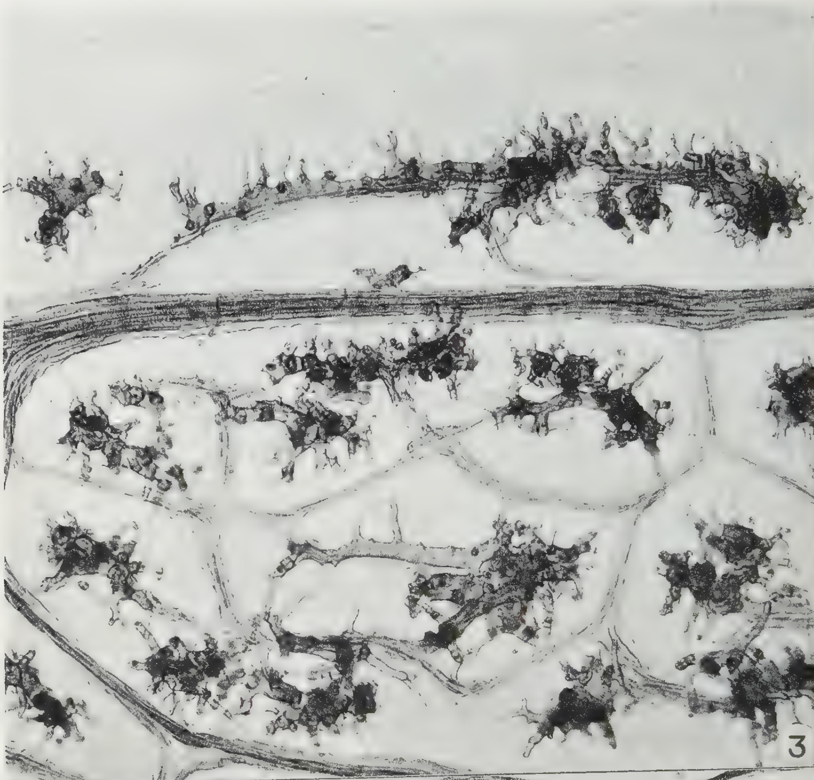
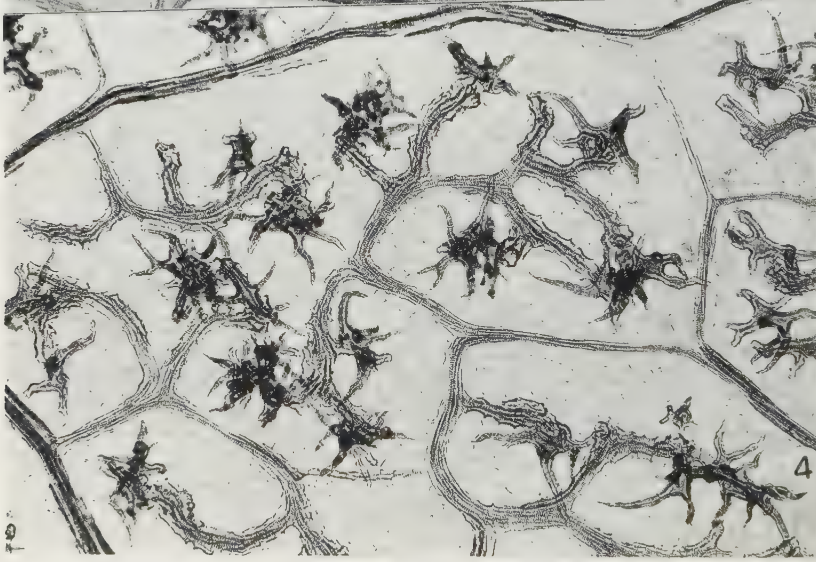




Foster, Foliar Sclereids in Boronella





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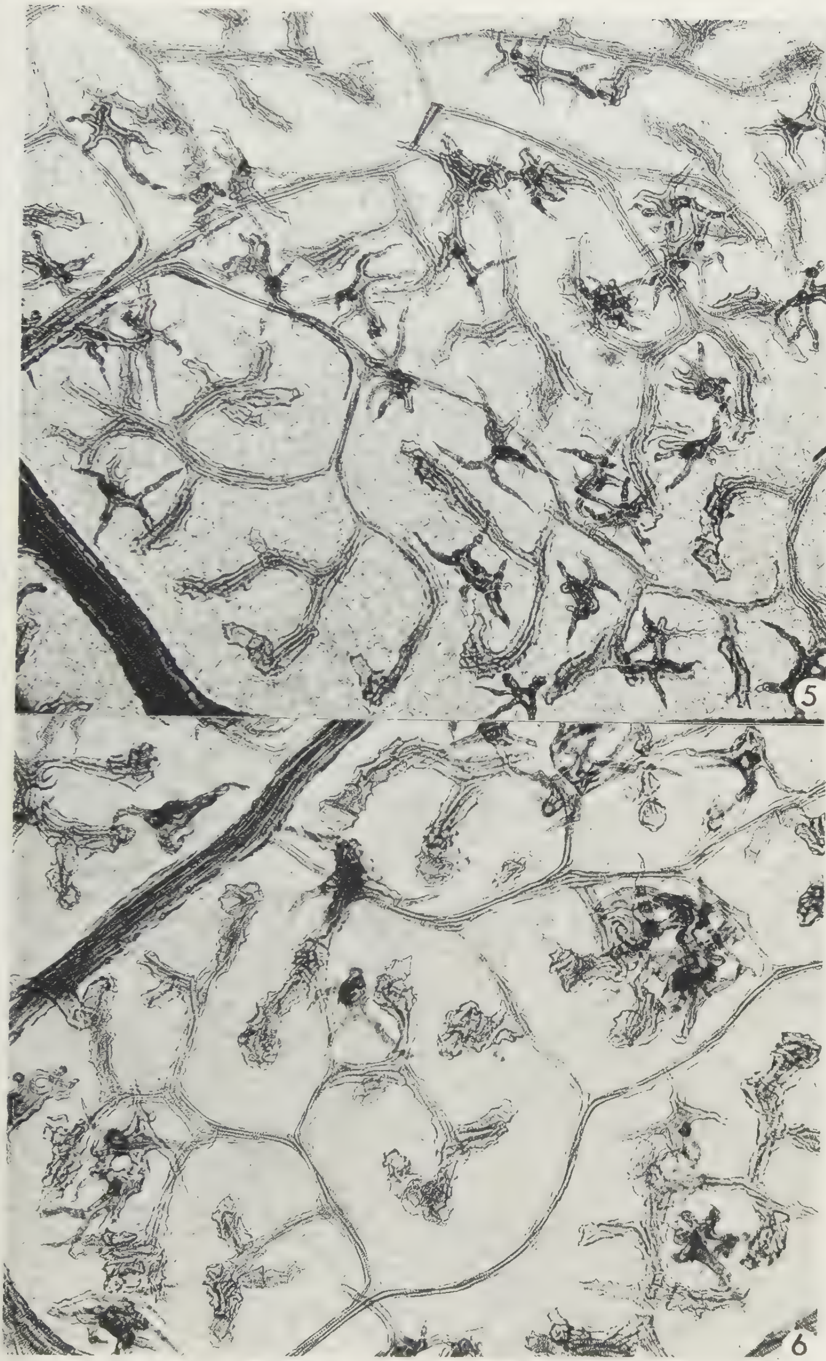

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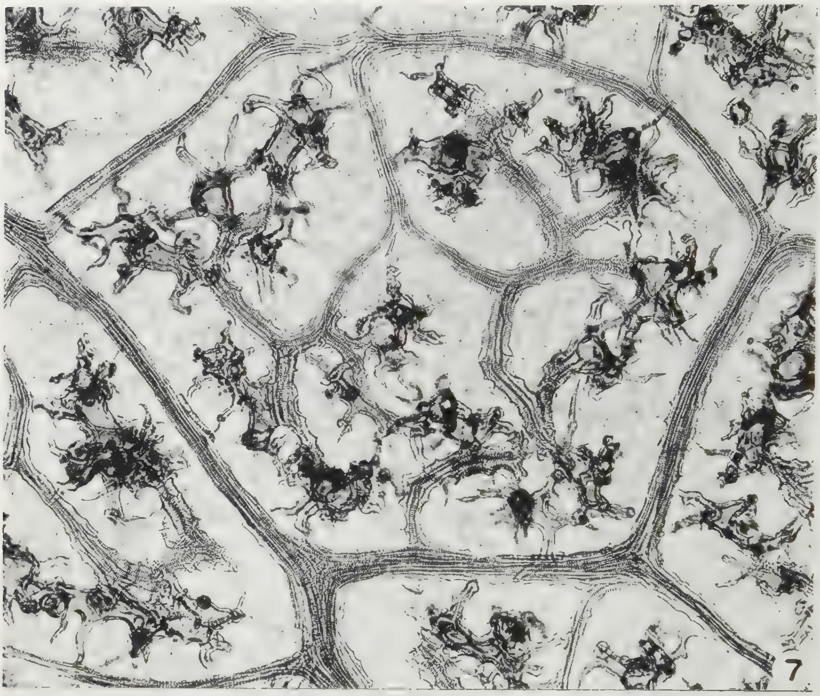

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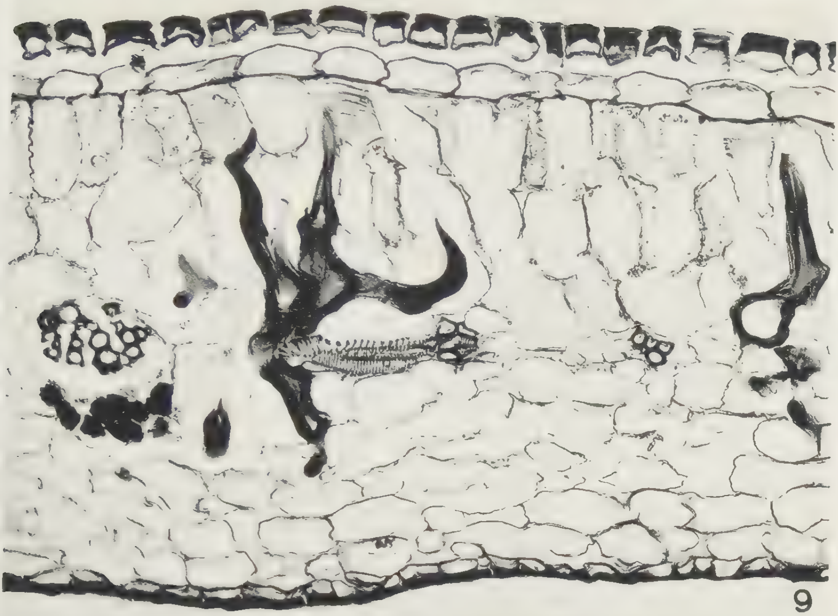

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FOSTER, FOLIAR SCLEREIDS IN BORONELLA

A TAXONOMIC REVISION OF PODOCARPUS IX. THE SOUTH PACIFIC SPECIES OF SECTION EUPODOCARPUS, SUBSECTION F

NETTA E. GRAY *

With one plate

SECTION *Eupodocarpus* as described in the first paper of this series (1) consisted of only four subsections; a fifth was added later (3) for an African species. Three species have subsequently appeared which, upon investigation of the leaf anatomy, require the addition of a new subsection (F) to the section. From New Caledonia, *Podocarpus longefoliolatus* had been described by Pilger (5) but the only specimen in the United States at that time, *White 2037* in the Arnold Arboretum Herbarium, had not yet been identified as this species. *Podocarpus decumbens*, also from New Caledonia, was not collected until Chevalier and MacDaniels visited Montagne des Sources in 1949. *Podocarpus decipiens*, in the Fiji Islands, was found only after the examination of a large number of specimens determined by others to be either *P. neriifolius* or *P. elatus*. Differing from all other subsections of *Eupodocarpus*, the leaves have two or more resin canals above the vascular bundle (FIGS. 1–3). These are not closely associated with the transfusion tissue and in *Podocarpus decumbens* and *P. longefoliolatus* there are quite uniformly four. An additional pair of resin canals actually in the transfusion tissue is often found in *P. decipiens* (FIG. 4) as in subsection B (also South Pacific species in section *Eupodocarpus*) but these are considered to belong with those under the vascular bundle.

Although the two land areas of New Caledonia and Fiji are, geologically, both continental in type (4), they differ greatly in the number of genera and species of gymnosperms to be found in each. New Caledonia is very rich, with at least 37 species representing eleven genera. Few of the genera are monospecific and of the largest, *Araucaria* has had eight species described and *Podocarpus* twelve. In contrast, the Fiji Islands are poor in gymnosperms, having only nine species in four genera, *Podocarpus*, *Agathis*, *Dacrydium* and *Acmopyle*, all of which are also in New Caledonia. Again *Podocarpus* is the largest with five species. The three species of *Acmopyle*, a genus closely related to *Podocarpus*, are found only in New Caledonia and Fiji. In *Podocarpus*, five of the eight sections are found in New Caledonia but only three of these, *Dacrycarpus*, *Polypodiopsis* and *Eupodocarpus*, are also on the Fiji Islands. Of the species in section *Dacrycarpus*, *Podocarpus vieillardii* in New Caledonia is endemic, but *P. imbricatus* in the Fiji Islands is of wide distribution in the southwest Pacific, only not on

* The author wishes to express her great appreciation to Dr. Albert C. Smith, Curator in the United States National Herbarium, Smithsonian Institution, for kindly reading this manuscript and especially for his careful criticism of the Latin descriptions.

Australia nor New Caledonia. In section *Polypodiopsis* there are three species in New Caledonia, one species in northern South America, and one species in both Fiji and New Guinea. *Podocarpus vitiensis*, the latter, will probably appear on other Malaysian islands as more collecting is done. The Fiji Islands are the eastern limit of section *Eupodocarpus*.* In subsection B, to be described later, *Podocarpus sylvestris* and *P. novae-caledoniae* are endemic in New Caledonia and *P. affinis* endemic in Fiji. *Podocarpus neriifolius*, also in Fiji, has, on the contrary, what is probably the widest distribution of any species in the genus: from the mountains of Nepal east into China and the Philippines, into southeastern Asia and the vast area of the Malaysian islands, but not Australia nor New Caledonia. Subsection F is found only with the endemic species *Podocarpus longefoliolatus* and *P. decumbens* in New Caledonia and *P. decipiens* in Fiji.

The distribution of these species in New Caledonia and Fiji only is not common. Smith (7), in his studies of the Fijian flora, calculates that only 5.3% of the endemic species of phanerogams have their nearest relatives in New Caledonia or Australia. He names *Kermadecia* as an example of a genus known only from New Caledonia and with two species in the Fiji Islands. That *Podocarpus imbricatus* and *P. neriifolius* are absent in New Caledonia but otherwise widely distributed, and that the species of subsection F are distinct and endemic in New Caledonia and Fiji would substantiate Smith's assumption that "the last land connections to be sundered in this general region were those joining Fiji with the New Hebrides, the Solomons, and New Guinea" and "land connections between Fiji and New Caledonia were disturbed much earlier."

Podocarpus longefoliolatus, *P. decumbens* and *P. decipiens*, the species in this subsection, have two subulate bracts beneath the receptacle in the female cones and do not have the Florin ring in the subsidiary cells surrounding the stomata, characters which heretofore have been considered to distinguish subsection B from the others in *Eupodocarpus*. These characters are not common to all of the section *Eupodocarpus* in the land regions of the southwest Pacific, as the species assembled in subsection D, one of which is found in New Caledonia, one in Australia and Tasmania and the rest in New Zealand, do not have the bracts and do have the Florin ring.

Transverse sections of the leaves in subsection F show the single vascular bundle, hypodermal fibers, transfusion tissue and accessory transfusion tissue, upper palisade parenchyma and stomata only on the lower side which are characteristic of all members of *Eupodocarpus*. The lack of marginal resin canals and the Florin ring in the subsidiary cells surrounding the stomata are characters found only otherwise in subsection B of this section. Characteristics common to only some of the species in subsection B are auxiliary sclereids in the spongy and palisade parenchyma, fibers in the regions above and below the vascular bundle, interrupted hypoderm, and occasional fibers between the stomatal rows on the lower side. The two or more resin canals above the vascular bundle, distinguishing subsection

* Recent collections extend section *Eupodocarpus*, subsection F, into the Tonga Islands.

F from the others, were not noted by Orr (5) in his examination of *Podocarpus longefoliolatus*.

Podocarpus longefoliolatus Pilger in Pflanzenreich **IV. 5**(Heft 18): 79. 1903, in Nat. Pflanzenfam. ed. 2, **13**: 248. 1926; Guillaumin in Bull. Mus. Hist. Nat. Paris **18**: 100. 1912; Compton in Jour. Linn. Soc. Bot. **45**: 426. 1922; White ex Wilson & Guillaumin in Jour. Arnold Arb. **7**: 79. 1926; Florin in Svenska Vet.-Akad. Handl. ser. 3, **10**: 279. 1931.

A small tree attaining 10–12 m. in height and 50 cm. in diameter; bark reddish and longitudinally furrowed, its outer layers sometimes grayish; branchlets thick with usually crowded leaves, the young stem ridged and furrowed from the base of the leaf petioles, the leaf scars round and flat on the older part of the twig. Terminal buds elongate with long-acuminate scales arising from a broad base, the outer ones spreading and becoming foliaceous and recurved, up to 10 mm. long. Leaves remaining on the twig for several years, coriaceous, patent to erect, straight or subfalcate, narrowly lanceolate, gradually narrowing at the base to a short, thick petiole, angustate toward the tip, acute to obtuse at the apex, sometimes shiny above, duller beneath, revolute at the margin when dry, 5–10.5 cm. long, 6–10.5 mm. wide; midrib broadly prominent or irregularly ridged above, scarcely distinct or only shallowly impressed below. Transverse sections of the leaves (FIG. 1) show interrupted hypoderm and 4 resin canals above the vascular bundle with three below. Pollen cones solitary, sessile, cylindric, 1.5 cm. long and 3 mm. wide, the scales narrowly triangular, the outer spreading, recurved and keeled. Microsporophyll tipped with short, thick, slightly incurved apiculus. Ovulate cones solitary in the axils of the leaves of the new growth; peduncle 1–2 mm. thick, angled, 1–1.7 cm. long; receptacle fleshy, about 8 mm. long, of 3 fleshy scales fused most of their length, free and spreading at the tips, subtended by 2 thin subulate bracts about 5 mm. long usually deciduous by maturity; bearing 1–3 (usually 2) ovules, bluish and glaucous when young. Seeds ovoid, 9 mm. long, 7 mm. wide, scarcely crested at the apex.

DISTRIBUTION: New Caledonia, found only certainly in rain forest on summit of Mt. Mou, 1000–1100 m. elevation, and perhaps on the nearby Isle des Pins.

SPECIMENS EXAMINED: *

NEW CALEDONIA: Mt. Mou, *Pancher s.n.* (type coll., P, 2 sheets, Herb. at Noumea), *Vieillard 1265* (BM; P, 2 sheets), *Buchholz 1098* († Ill), *1446* (A, † Ill), *1584* (Ill), *1682* (Ill), *1792* († Ill), *White 2037* († A), *MacDaniels 2324* († CU). LOCALITY UNCERTAIN: Isle de Pins, *Pancher V1265* († ♀P).

* The following symbols indicate the herbaria having the specimens cited: Arnold Arboretum (A), Bernice P. Bishop Museum in Honolulu (Bish), British Museum (BM), University of California at Berkeley (UC), Chicago Natural History Museum (Field Museum) (F), Cornell University (CU), Gray Herbarium (GH), University of Illinois Herbarium (Ill), New York Botanical Garden (NY), Paris Museum Herbarium (P), Stanford University Dudley Herbarium (DS).

† This symbol preceding the abbreviated name of an herbarium following the

This species was described by Pilger (6) from Pancher and Vieillard collections in the Paris Museum Herbarium which he found filed with *Podocarpus novae-caledoniae.* Foliage of *Podocarpus longefoliolatus* has been confused with *P. sylvestris* Buchholz, belonging to subsection B. The terminal buds of *Podocarpus sylvestris* are ovoid and differ in the thinner, closely appressed scales. Also *Podocarpus sylvestris* is found in different areas at altitudes below 500 m. The leaf transverse section shows only the three resin canals below the vascular bundle.

It is not certain if three specimens seen by Prof. Buchholz *in the Herbarium of New Caledonia at Noumea are this species (*Vieillard 1275* from Bae du Sud, *Vieillard 1275* from Wagep and *Vieillard 1266* from Canala). Since these collections are not from the type locality of Mt. Mou where the only certain identification has been made, these must be excluded until further examination is possible. Pilger (6) listed the *Vieillard 1266* from the Paris Museum but since Vieillard repeated his numbers for different collections the Noumea specimen must still be questioned. Compton did not collect *Podocarpus longefoliolatus* from Isle de Pins. A female specimen in the Paris Museum, *Pancher V1265,* is *Podocarpus longefoliolatus*, but the locality of Isle de Pins has been questioned.

The transverse sections of the leaves of this species show a thick cuticle and small vascular fibers are often abundant between the resin canals below the phloem but rare above the vascular bundle. The auxiliary sclereids present in the mesophyll are common in leaves more than one year old. Sometimes the palisade layer is more than one cell thick. The hypodermal fibers are usually in small groups on the upper surface of the leaf and are very rarely found between the stomatal rows on the lower surface. A 3-months-old leaf, from the specimen *Buchholz 1446*, gave some interesting light on the ontogeny of tissues and structures in leaves of *Podocarpus longefoliolatus.* The epidermis, transfusion tissue, and resin canals were fully mature. The xylem and phloem did not show complete cell wall thickening. The accessory transfusion tissue was remarkable in that it appeared so completely crushed that the cells could not be distinguished. Hypoderm and palisade parenchyma were not differentiated although many of the cells were already present. The mesophyll cells were large and were not crushed even though there was no apparent thickening of the cell walls. There were no vascular fibers.

Podocarpus decumbens sp. nov.

Frutex plerumque humilis 30–40 cm. altus, caudice primo 4–5 cm. diametro, ramis decumbentibus 2–3 cm. diametro interdum radicantibus, ramulis adscendentibus crassis glaucis, cum foliis confertis interdum

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specimens examined signifies that the details of the leaves of this specimen have been examined in cross-section.

* From notes made by Prof. John T. Buchholz on his collecting expedition to New Caledonia 1947–48.

verticillatis, e basi petiolorum striatis et canaliculatis; alabastris ovatis, squamis longe acuminatis 6–10 mm. longis, vel exterioribus carinatis apice crassis et margine inferno erosis, aliquando demum foliaceis ad 18 mm. longis, squamis interiorbus triangularibus 3 mm. longis; foliis coriaceis, ex linearibus oblanceolatis, 3–5.8 cm. longis, 4–7 mm. latis, supra nitidis subtus glaucis apice obtusis, basi in petiolum brevem crassum angustatis; costa supra angusta et manifesta, subtus lata (at 1.5 mm.) et striata; foliis semper cum ductis resiniferis 3 sub vasi phloemate, 4 vel plus supra xylum; strobilis masculis ex alabastris sessilibus axillaribus, 1.5–2 mm. longis, squamis latis obtusis, cylindricis, 1.5–3 cm. longis, 3 mm. crassis, squamis demum late deltoideis, carinatis, scariosis et laciniosis, apice acutis; microsporophyllis 1.8 mm. longis, conferte imbricatis, apice crassis, fusco brunneis; strobilis foemineis et seminibus ignotis.

DISTRIBUTION: New Caledonia, only from Montagne des Sources, 900 m. alt., growing under forest among ferns and *Dracophyllum*.

SPECIMENS EXAMINED:

NEW CALEDONIA, Montagne des Sources, *Chevalier* in 1949 (TYPE † Ill), *MacDaniels 2379* († CU).

The description of *Podocarpus decumbens* was written from the specimen collected by Luc Chevalier on Dec. 1, 1949, on his trip with Prof. L. H. MacDaniels who also collected a specimen which I have examined. The small male cones have fallen from the specimen but they are mature with open microsporangia and the basal scales are still on the twig where they were attached. The very low creeping habit is rare in this genus of trees but it is interesting to note that *Podocarpus gnidioides*, in subsection D, of similar habit and only a little larger, is also found on Montagne des Sources. There is no confusing these species in the field or herbarium as the foliage is very different, the leaves of *Podocarpus gnidioides* being only up to 2 cm. long and 2 mm. broad. Only the sketch made by M. Chevalier convinces me that the twig with such large leaves, up to 5.8 cm. long and 7 mm. wide, came from so small a mature plant.

Transverse sections of the leaf of *Podocarpus decumbens* (FIG. 2) show a small vascular bundle with lateral wings of transfusion tissue more than half as wide as the bundle. There are three resin canals below the vascular bundle, the two lateral often near the outer extensions of the transfusion tissue, variable in size. There are two to four resin canals above the vascular bundle, the outer two often located near the inner edge of the transfusion tissue and also variable in size. The accessory transfusion tissue sclereids have somewhat irregular shapes and larger intercellular spaces. The hypoderm is interrupted above (the fibers large) and is rarely doubled; and is absent below between the stomatal rows. Vascular fibers are abundant between the phloem and resin canals and rare above the vascular bundle. Auxiliary sclereids are abundant in the mesophyll. The palisade is of only one layer of cells and is interrupted by the occasional auxiliary sclereids. A single layer of cells similar in shape, though shorter, is arranged like palisade tissue on the lower side of the leaf. Epidermal cells

are one-third to one-half larger in *Podocarpus decumbens* than in *P. longefoliolatus*.

Podocarpus decipiens sp. nov.

Arbor dioica ad 20 m. alta, plerumque minor raro fruticosa, cortice pallido, ramulis divaricatis; alabastri terminalis squamis crassis ovatis, exterioribus longe attenuatis, interioribus brevioribus apiculatis; foliis coriaceis levibus utrinque nitidis, subtus subfuscis, lineari-lanceolatis, interdum subfalcatis, fastigio supra costam plerumque complanato subtus suppresso, 6.3–13 cm. longis, 7–16 mm. latis, apice late acutis vel acuminatis, basi in petiolum 6–12 mm. longum sensim angustatis; foliis semper cum ductis resiniferis 3 sub vasi phloemate, 2 vel plus supra xylum; strobilis masculis cylindricis subsessilibus axillaribus interdum fasciculatis, ad 5 mm. longis et crassis, squamulis numerosis valde carinatis basi circumdatis, saepe longe apiculatis (si pluribus in fasciculis propter ramulos intra basim); microsporophyllis apiculo longo angusto ornatis; strobilis foemineis axillaribus solitariis, pedunculo angulato 5–15 mm. longo enatis, maturitate puniceis; receptaculo squamis multibus coalescentibus 5 mm. longis composito, saepe 2-ovulato, bracteolis 2 membranaceis gracilibus 3–5 mm. longis basi subtento; semine glauco subgloboso, 10 mm. longo, 8 mm. lato, interdum obtuse cristato, inferne haud angustato.

DISTRIBUTION: In the Fiji Islands, on both Viti Levu and Vanua Levu, usually at altitudes over 700 m. on mountain slopes and summits.

SPECIMENS EXAMINED:

FIJI: Viti Levu: MBA: Mt. Ndelaiyoö, on the escarpment west of Nandarivatu, *Smith 5075* (♂ and ♀) (TYPE † Ill) (A); 2 mi. down Mba Trail from Nandarivatu on Malawathi Creek, *Gillespie 4227* († Bish, UC); Mt. Koroyanitu, Mt. Evans range, *Smith 4116* (A, † Ill); Mt. Nairosa, Mt. Evans range, *Smith 4092* (A, † Ill); between Nggaliwana and Tumbeindreketi Creeks, east of Navai, *Smith 6000* (A, † Ill), *Smith 5992* (A, † Ill); vicinity of Nandarivatu, *Gillespie 4034* († Bish, UC), *Gillespie 4203* († Bish, † DS, UC); NAITASIRI: vicinity of Nasinu, 9 mi. from Suva, *Gillespie 3444* († Bish, UC); REWA: Quarry beyond Lami village, *Gillespie 4609* († Bish); Mt. Korombamba, *Parks 20146* († Bish, UC); NAMOSI: *Parks 20197* († Bish, UC). Vanua Levu: THAKAUNDROVE: Yanawai river region, Mt. Kasi, *Smith 1769* († Bish, †F, GH, † NY, UC). Locality uncertain: Ra(?): *Degener 15565* († A).

Podocarpus decipiens is usually a tree, often attaining sufficient size to be used in the lumber industry; it is called "asimbolo" throughout Viti Levu, but in the Mt. Evans region was noted as "ngali." On Vanua Levu it was recorded under the name of "mbau." It most frequently grows in dense forests on slopes and summits of mountains at altitudes over 700 m. where the angiosperm species, *Degeneria vitiensis*, was sought for and found much more abundantly than expected. However, *Gillespie 4609* was found growing in limestone rocks only about 6 m. above a tidal swamp in Rewa Province, and *Gillespie 3444* at 150 m. in southern Naitasiri Province. *Parks 20197* is said to be from a shrub 1–2 m. high, suggesting

a possible habit similar to *Podocarpus decumbens* in New Caledonia, but since it is sterile and has leaves about four times the size of *P. decumbens* I prefer to include it as a juvenile specimen of *P. decipiens*. Gillespie did not indicate the shrubby or arboreal habit of his specimens. The outer terminal bud scales are usually long with attenuate, often foliaceous tips; *Gillespie 4609* shows an extreme in its broadly ovate bud scales with long apiculate apices. *Degener 15565, Gillespie 4609* and *Smith 5075, 4116*, and *4092* do not have leaves more than 10 mm. wide. The remaining specimens have broader leaves (*Smith 6000* being the broadest with leaves up to 23 mm. wide) and usually come from the lower altitudes of the range. Most of the specimens have leaves with a broad, only slightly prominent midrib. This is in contrast to the abruptly prominent midrib in *Podocarpus neriifolius*. The margins of the leaves are not revolute.

Wasscher's recent interpretation of *Podocarpus neriifolius* has so much broadened the description that the usually thinner leaves (a most indefinite character) is the only external distinguishing feature. Both species grow in Fiji, but *Podocarpus decipiens* grows at higher altitudes and has the resin canals above the vascular bundle in a transverse section of the leaves. Hybridization between the two species no doubt occurs and this accounts for the difficulty in making a positive determination for some of the specimens.

Studies in the leaf anatomy of *Podocarpus decipiens* (FIG. 3) show an interrupted hypoderm of small fibers, 16–29 μ, similar to *P. neriifolius*. Occasionally a few lower hypodermal fibers have been found between the stomatal rows. The resin canals above the vascular bundle is a character distinguishing it from other species of *Podocarpus* found in the Fiji Islands. Below the vascular bundle there are three or five resin canals and above the bundle the number may vary from two to six. A total of as many as nine resin canals encircling the vascular bundle has been observed, and one pair may be found actually within the transfusion tissue (FIG. 4). In *Smith 4092, 5992* and *Gillespie 4227* no upper resin canals have been found. Auxiliary sclereids in the palisade and spongy mesophyll are sometimes quite abundant and at other times very rare and cannot be used as a distinguishing character. The transfusion tissue and accessory transfusion tissue are uniform and well-developed.

Smith 4116 is atypical in the early deciduous bracts below the receptacle in the young stage and the exceptionally long peduncle (9–25 mm.).

Although I did not have the opportunity to examine it, *Gibbs 743*, from Nandarivatu, at 900 m., is probably *Podocarpus decipiens* instead of *P. neriifolius* as suggested. The site of the collection (*2*) is the same as that of the type, *Smith 5075* (*8*). *Gibbs 819* (*2*), said to be from a "slender erect tree, general in the forest" at altitudes over 900 m., may also be this species. It definitely is not *Podocarpus elata* R. Br., as identified, for this species has not been found outside of Australia except in cultivation.

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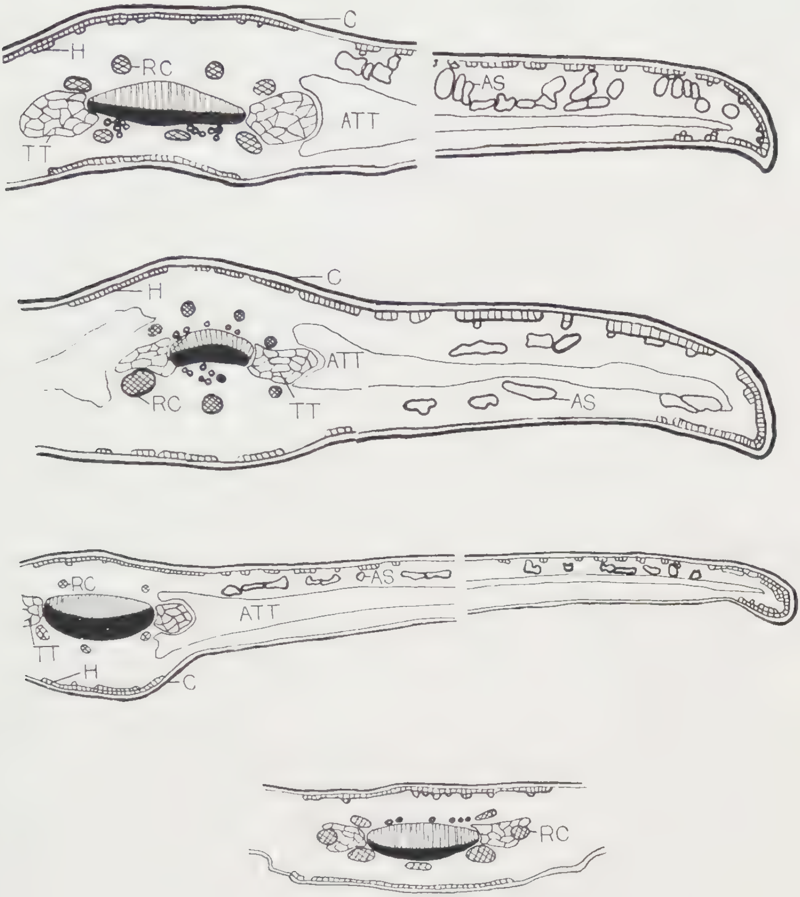
EXPLANATION OF PLATE I

FIGURE 1. *Podocarpus longefoliolatus* Pilger. Transverse section of leaf, *MacDaniels 2324*, showing relative thickness of (C) cuticle, extent of (H) hypodermal fibers, location of (RC) vascular resin canals with 4 above the vascular bundle, (TT) transfusion tissue, (ATT) accessory transfusion tissue, and (AS) auxiliary sclereids in the mesophyll. × 20.

FIGURE 2. *Podocarpus decumbens* Gray. Transverse section of leaf, *MacDaniels 2379*, showing same tissues as FIG. 1, with 4 resin canals above the vascular bundle. × 20.

FIGURE 3. *Podocarpus decipiens* Gray. Transverse section of leaf, *Degener 15565*, showing same tissues as FIG. 1, with only 2 resin canals above the vascular bundle. × 20.

FIGURE 4. *Podocarpus decipiens* Gray. Transverse section of leaf, *Smith 5075*, showing only the tissues in the region of the vascular bundle, with resin canals in the transfusion tissue as well as 2 above and 3 below the vascular bundle. × 20.



GRAY, PODOCARPUS

THE VEGETATION OF BEATA AND ALTA VELA ISLANDS, HISPANIOLA

Richard A. Howard *

With five plates

Perhaps the least explored area of the Greater Antilles is the southern promontory of the Dominican Republic, the province of Barahona. Only one unimproved road extends across this low-lying, triangular-shaped, dry waste of Eocene and Pleistocene limestone and through the thorn shrub forests and brush lands. Only the collections of Nash and Taylor, Ekman, and Howard are available from this area, and these represent scattered locations and incomplete records of a promising flora rich in dry-land and desert endemics. Off the southern tip of this promontory are two small islands, Beata and Alta Vela, which are the basis for this study. Beata and Alta Vela, geologically related to the tip of Barahona peninsula, are intriguing small bits of land in the Antilles. Both are important in the role they have played in the history of the Caribbean. Beata was visited casually twice by botanists, and until the occasion of my trip there, Alta Vela was unrepresented in the botanical records of the Antilles.

I had seen these two islands several times from the sea, land, and air. My desire to visit them was accentuated by the fact that Erik Ekman, perhaps the most thorough of botanical collectors who have visited Hispaniola, had never been on these islands. During his extensive stay in Haiti and the Dominican Republic Ekman visited many of the islands adjacent to this larger land mass. He published comprehensive studies of the vegetation on Los Siete Hermanos, Tortue, Gonave, and Navassa, and found on each richness of endemics and significant information on the relationships of the floristic entities in Hispaniola. Beata and Alta Vela, lying as they do, should contribute additional information in the phytogeographic puzzles of the southern peninsula of Haiti and the Barahona coastal ranges.

Thus, in planning a visit to the Dominican Republic in 1950, I wrote in advance to the government of the Dominican Republic for permission to visit Beata and Alta Vela. This was necessary because Beata is a naval station of the armed forces of the Dominican Republic, which also maintain on Alta Vela a lighthouse. My request was referred to Mr. Manuel de Moya, then Secretary of Agriculture, who took prompt action to grant my request and to expedite my visit to these islands. Instructions were given

* The botanical observations and the collections which form the basis for this paper were made in 1950 when I had the opportunity of spending seven months in the Caribbean Islands. This extensive trip was made possible financially by grants from the Penrose Fund of the American Philosophical Society and the Milton Fund of Harvard University. I am particularly grateful for this support.

to the commander of the destroyer escort vessel "27 de Febrero" to call at the Port of Barahona and to supply transportation as I wished. It is difficult to express adequately my gratitude to Mr. Moya and to the commander and men of this vessel for their complete cooperation in making this botanical expedition possible.

We left Barahona on August 7th, making the run to Beata in a few hours. This in itself stands in contrast to the visit Alexander Wetmore made to Beata in 1929, when he rented a sailboat and spent twenty-four hours getting from Barahona to Beata and three days on the return voyage. The crew of the "27 de Febrero" supplied transportation from the anchorage in the northwest corner of the island several times daily to shore and back to the boat. A cot was supplied me when I chose to sleep on deck rather than in the officers' quarters in the shorter bunk which had been offered below deck. The meals were those of the officers' mess and were rich with the natural products of the country and the sea and beaches of and around Beata. Plantains, cassava, and other root crops were supplemented and augmented with a great variety of sea food from the Caribbean, turtle steaks from animals we netted or caught on the beaches, and goats which the crew shot in the thorn scrub of Beata. Each day the ship lifted anchor and took me to a different section of the Beata shore line to explore inward, and a one-day trip was made to Alta Vela before the week I had allotted was up and it was necessary to return to Barahona. I enjoyed the hospitality and courtesy of the Dominican Navy and the company of the crew on many trips into the difficult vegetation of these islands.

Beata Island was apparently first seen by Columbus on his second voyage. On his third voyage, in 1498, Columbus is reported to have arrived at his former anchorage under the little island of Beata on the twentieth of August.

The first complete description of Beata appears in a report of the U. S. Coast Survey in Senate Executive Document No. 38 of the Fortieth Congress, 1868. This report considers both Beata and Alta Vela and mentions that "Beata island, the larger of the two, lies four miles south by east from Beata point, the southernmost extension of Barahona peninsula." The island is described as "five miles long and two miles broad mostly covered with brushwood from 50–80 feet high. The southern part is the most elevated and the northern end terminates in a long low point. Off the west point there is no bottom to 136 fathoms of line three quarters of a mile distant. The east side is very deep and the north and northeast sides connect to Beata point by a shallow white bank with the greatest depth being three fathoms."

Various reports of the richness and the utilization of Beata Island appear in the older literature and descriptions of the island, entirely unsupported by any evidence found on the island today. Moreau de Saint-Méry in 1796 described the abundance and quality of the wood on Beata, which indicated the high fertility of the land. Additional comment was made on the increase in the size of the herds on the island. Moreau felt it possible to establish estates with flocks of sheep, as existed in former times. James

Playfair in his *Geography* (6: 625. 1814) states, "On Beata the soil is good and was once in a state of cultivation. Till the fall of Santo Domingo, it was the great resort of French privateers." As recently as 1918 Otto Schoenrich (*Santo Domingo, A Country with a Future*, pages 113–114) described Beata as follows: "The island is covered with dense forests in which wild cattle abound. During the sixteenth and seventeenth centuries the island was a convenient resort for the pirates that infested the Spanish main; at one time it is said to have contained fine plantations, but at the present it is only occasionally visited by Dominican or Haitian fishermen." These reports are hard to believe in the light of a present-day examination of the island.

Only two modern visits to the island have been made by botanists, to the best of my knowledge, although zoologists and anthropologists have been there more frequently. The Danish Oceanographical Expedition with the steamer Dana explored the warmer parts of the Caribbean Sea and the Gulf of Panama in 1921 and 1922. Strong trade winds forced this ship to seek shelter on the west side of Beata on Feb. 22, 1922, and apparently it was anchored in essentially the same spot that Columbus chose. One of the botanists aboard this ship was C. H. Ostenfeld, who took advantage of the two-day lay-over to collect marine algae and to make a short visit ashore. The specimens of flowering plants which he collected were described by Urban in the *Dansk Botanisk Arkiv* 4(7): 1–37. 1924. Ostenfeld reports that they landed at the northwest corner of the island. "No inhabited human dwellings were to be seen but a grassy area mostly covered with *Panicum* (*maximum*?) and scattered cotton plants shew that formerly culture had existed here." Ostenfeld indicated throughout his brief report that the island was a difficult place to travel on or to collect plants.

Between 1929 and 1934 the expeditions sponsored by Allison Armour on his yacht the Utowana made several stops at Beata Island. I have been unable to date these specifically and have been able to locate only four records of plants collected during these visits. Thomas Barbour in his privately printed book, "Allison Armour and the Utowana," refers to his visits to Beata aboard the Utowana in the spring of 1929 and again in April of 1934. In 1932 the Utowana apparently made another visit to Beata Island, for one of the plants collected by the botanists aboard, David Fairchild and P. H. Dorsett, proved to be new and was described as a new genus *Armouria* (= *Ulbrichia*) of the Malvaceae. Mr. Harold Loomis, director of the Plant Introduction Station of the U. S. Department of Agriculture at Coconut Grove, Florida, was also on this expedition and introduced seeds of this new genus for cultivation in Florida. David Rogers, in his revision of *Stegnosperma* of the Phytolaccaceae, cites two other collections from Beata Island by these men. No collectors' lists or field books have been located for this expedition, although a gap of twelve numbers occurs between the *Stegnosperma* and *Armouria* collections.*

* After this paper was prepared I had the opportunity of visiting Mrs. David Fairchild at Coconut Grove and Mr. Harold Loomis at the Plant Introduction Station, also in Coconut Grove, Florida. Mrs. Fairchild kindly made Dr. David Fairchild's

Herbert W. Krieger, in an article on the Culture Sequences in Haiti (Smithsonian Institution Publication 3134: 113–124. 1932) refers to Beata Island and publishes a photograph of the shell mounds of pure *Strombus gigas* on the island. I was unable to determine the source of this photograph or the nature of the expedition. Alexander Wetmore visited Beata for a time in the spring of 1931, as he reports in the Smithsonian Institution Publication 3134: 45–54. 1932. He and his party hired a sail-boat and sailed for Barahona. Wetmore illustrated a small settlement called Ocrik on Beata Island and reported that his party stayed in one of the houses. The settlement at Ocrik was not in evidence during my visit in 1950. Wetmore's description of the vegetation indicated that it has changed little since Ostenfeld's visit, as he reports, "Thorny bushes, trees and vines growing from the scanty soil accumulated in crevices in the rock form a jungle so dense that it may be penetrated only along trails cut laboriously into the interior."

Thus I seem to be the first botanist to make a deliberate attempt to visit this island and to make comprehensive collections. I am not satisfied

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field notes for this expedition available to me for study. She was also able to refer to photographs made on Beata Island, and her recollections of the Utowana's stay at Beata were most pleasant and helpful. Mr. Loomis, also, was able to refer to field books for information on his collections as a member of the party, and he had some excellent photographs of the area. From the field notes of Dr. Fairchild and Mr. Loomis I was able to prepare a numerical list of their collections on Beata, and Mr. E. C. Leonard of the U. S. National Herbarium has been most helpful in checking the herbarium for these specimens. He was not able to locate all of them, and in some cases in the following list field determinations are given. I am most indebted to Mrs. Fairchild, Mr. Loomis, and Mr. Leonard for their assistance.

The Utowana, with Fairchild, Dorsett and Loomis as botanists, stopped at Beata Island on January 17th and March 22nd, 1932. Only a single collection was made by Loomis on the latter date. He collected six numbers on the island on January 17th. Number 15 was identified in the field as *Coccothrinax ekmanii*. This is currently called *Haitiella ekmanii*. The specimen was not located in the National Herbarium. Numbers 16, 17, 18, 19 and 20 collected by Mr. Loomis were species of *Gossypium*. No material could be located at the National Herbarium. On March 22, 1932, Mr. Loomis collected a single number, 94, representing *Pseudophoenix saonae*. Mr. Loomis stated that Cook was in doubt regarding this identification at the time, but Mr. Leonard reports that a specimen so identified is in the National Herbarium.

The following collections by Fairchild and Dorsett were all made on Beata Island on January 17, 1932. Specimens present in the National Herbarium are indicated with an asterisk. All other names represent field identifications from Dr. Fairchild's field notebooks: 2603, *Guilandina ovalifolia*; 2604, *Opuntia moniliformis*; 2605, *Stegnosperma cubense**; 2606, *Stegnosperma cubense**. Number 2606 in Fairchild's field notebook is identified as *Zanthoxylum* sp. A specimen with the same number is cited by Rogers and is in the National Herbarium as *Stegnosperma cubense*. No. 2607, *Capparis flexuosa**; 2608, *Plumeria obtusa* var. *obtusa**; 2609, *Tribulus cistoides*; 2610, *Bromelia* sp.; 2611, *Exogonium eriospermum**; 2612, *Canavalia maritima**; 2613, *Maytenus reynosioides**; 2614, *Coccothrinax ekmanii*; 2615, *Rhynchosia minima**; 2616, *Indigofera suffruticosa**; 2617, *Ulbrichia beatensis**.

One addition can be made to the list of species from Beata Island given in this paper, on the basis of the Fairchild and Dorsett collections available in the National Herbarium. *Rhynchosia minima* (L.) DC. should be added to the Leguminosae.

that I have mastered the flora, however, and wish to pay a return visit at a different season.

Beata today appears from the sea to be a low flat island (FIG. 1). It is a large block of limestone which apparently has been tilted by coastal adjustments. The island is approximately five miles long and three miles broad, according to the skipper of the "27 de Febrero." It is shaped like a wedge of pie, broadest at the northern end and slightly rounded at the south. The north end (FIG. 3) is a sandy plain, at sea level, interrupted by shallow bays now filled with marine succulents and mangroves. The eastern side, likewise, is mostly level with the sea. The greatest elevation is approximately three hundred feet, at the southwest corner. The entire western side of the island shows a series of wave-cut benches with an escarpment of fifty to two hundred feet running from the coast at the north to a point well inland at the southern end of the island (FIG. 2). The surface of this major block is essentially flat, tilting slightly to the northeast. It appears, therefore, that this block of limestone has been tilted by sinkings on the northern and eastern edges. The first crustal adjustment was the most severe, but evidence of at least two other benches on the western side indicates subsequent changes in the attitude of the limestone.

Ostenfeld described Beata as "consisting of rocks of volcanic origin, being more or less horizontally lying light-colored lava-beds. There is often a short terrace of bare lava-rock; from it the inclination towards the water is nearly vertical, often interrupted by fissures and small caves into which the waves break with much noise." He made mention of the lush green vegetation on the coastal sand, accumulations of *Sesuvium portulacastrum*. Inland from the shore Ostenfeld noted that the most favorable place for the vegetation was in the lee of cliff faces. Above this was the flat but faintly sloping plateau. Ostenfeld climbed the cliff face and found on top a plateau covered with much wind-blown vegetation of low, many-branched trees. He comments, "Many lianas interweave the small trees and numerous cacti and other shrubs are scattered amongst the trees. It was very difficult to make a way through this vegetation, also because it was without any shade at all and extremely hot. The soil surface was nearly bare lava-rock which gave a sonorous sound when we walked on it, and loose soil was found in small holes and deepenings. It was really quite astonishing to find such a comparatively dense plant cover on this rocky surface." Further in his report Ostenfeld relates, "The difficulty in walking in this shade-less vegetation of 3–6 meters high trees, hindered by lianes and the stinging Cacti and other thorny shrubs, prevented me from proceeding far into it. At a distance some slender palms — *Thrinax* or another genus of the same appearance — rose above the low trees, but I could not reach into them. Therefore, I turned back, climbed down the steep cliff-wall to the shore-terrace and examined the vegetation sheltered by the wall." Ostenfeld concludes his report by stating, "Taken as a whole the vegetation shows that the climate must be very dry and probably the rainfall is very low, which together with the strong and constant wind produces the pronounced xeromorphism; but of course no meteorological

data are available. My visit was in winter which probably is the rainy season, but one did not get much impression of that, the only sign may be the shooting of new leaves on some of the *Bursera*-trees and the *Guaiacum*-shrubs."

I had the opportunity during my visit there to cover much more of the island than did Ostenfeld and to penetrate deeper into the spiny shrubland. I established as a starting point for most work the sandy beach area in the northwest corner of the island. This is the location that Ostenfeld mentioned where he saw a number of cotton plants, indicative to him of former cultivation. From this point I worked as far southward as possible on the western side of the island. I also penetrated directly eastward from that starting point and made a long trip around the northern end of the island, working due south from the north towards the interior, later continuing around the northern end and down the eastern side to a point beyond the abandoned salt ponds. Later, with the assistance of the destroyer escort, several landings were made along the west coast at favorable places, and penetration was made directly inland to the cliff face and onto the plateau in three locations.

Sea-level shore lines of Beata Island are of three types: sand, low limestone shelves or benches, and mangrove swamps. Small sandy beaches are common on the west coast (FIG. 2). One of these in the northwest corner of the island is now occupied by several buildings which form the on-shore headquarters of the naval units stationed at Beata. The sailors have attempted to grow some crop plants, but with little success, due to the low nutrients present in the sand and the lack of rainfall. With no accumulation of fresh water anywhere on the island, except in casual rock depressions, the buildings are used as catchments for rain water for the naval personnel. The largest sandy beach observed occurred on the northern end of the island, where on-shore winds have piled the sand on the outcrops of limestone rock.

The vegetation of the sandy beach areas is of the type found commonly in the Antilles (FIG. 2). *Panicum maximum*, an introduced grass, forms the largest stands of greatest height among the herbs. *Opuntia dillenii* occurs commonly at the edges of the grass. *Sesuvium portulacastrum* forms mats of succulent vegetation in open areas. Scattered and individual plants of *Tephrosia cinerea*, *Cenchrus pauciflorus*, *Cassia buchii*, *C. strigillosa*, *Corchorus hirsutus*, *Stachytarpheta jamaicensis*, *Indigofera suffruticosa*, and *Evolvulus alsinoides* occur on the open sand. Extensive development of the herbaceous vines *Ipomoea pes-caprae*, *Echites umbellata*, *Canavalia maritima*, *Passiflora suberosa*, and *Calonyction tuba* cross the open sand, and *Stegnosperma cubense* is rampant in protected areas near the stands of grass. To the rear of the open sand beaches, and especially in more sheltered areas, *Suriana maritima*, *Colubrina ferruginea*, *Lantana reticulata*, *Capparis flexuosa*, *Amyris elemifera*, *Jacquinia barbasco*, *Canella alba*, and *Caesalpinia anacantha* occur.

The limestone shelves are badly eroded and the surface is described as dogtooth limestone. Near the ocean the vegetation on these coastal shelves

is affected by the salt spray, about as described by Ostenfeld. In general all plants on these shelves are stunted and contorted. *Avicennia nitida, Amyris elemifera, Conocarpus erecta, Ernodea littoralis,* and *Rhacoma crossopetalum* are the most common woody plants. *Cyperus nanus, C. planifolius,* and *Lithophila muscoides* are the most common herbaceous plants on the limestone benches. Further inland on the limestone benches the effects of the salt spray and the wind are less evident, and the vegetation becomes more luxuriant, approaching in composition that of the plateau area. Evidence of repeated tilting of the island can be found on the western side, where several small cliff faces exist before the main break to the plateau is found. Each cliff face shows undercutting due to previous wave action. These small caves are devoid of vegetation and are extremely dry. It is these caves that seem to be the habitat of the rhinoceros iguana (*Cyclura cornuta*) near the sea.

At the northern end of the island the transition from sand to limestone is gradual and migratory due to the wind action of blowing sand from the beaches onto the gently sloping rock (Fig. 3). The typical succulent beach vegetation gives way to extensive stands of grass and sedge and finally to a belt of pure sedge growth at the edge of the limestone. One of the conspicuous areas of the sedge belt near the limestone was composed of a large stand of *Remirea maritima*. The white bracts of this plant attracted the eye from a considerable distance.

The outcrop of limestone rock at the northern end of the island, exposed as it is to strong winds and salt spray, was occupied by an open stand of shrubby plants (Fig. 4). The rock in this region was heavily creviced and broken. Many of the plants found there were rooted deep down in the cracks with only the crown of the plant protruding. The few plants which did root in the small soil pockets on the limestone were strongly wind-blown, often with branches developing only on the leeward side of the plant. A few species, *Clerodendron spinosum, Erithalis vaccinifolia, Antirrhoea elliptica, Strumpfia maritima, Borrichia arborescens,* were found in this area alone on the island. The concentration of the palm *Haitiella ekmanii* in this wind-blown shrub zone was also unusual. *Haitiella* was found commonly in the interior of the island on the limestone plateau, but as scattered or individual plants. In the northern shrub zone just mentioned, however, plants of *Haitiella* were clustered (Fig. 4).

The third coastal type of vegetation, the mangrove swamp formation, was also most common on the northern end of the island. The shallow coastal waters and the gradually ascending beach area have combined to produce the type of shore line best suited to mangrove development. Apparently the profile of the shore line has varied in the past, due to shifting currents and deposition and removal of soil. Some of the mangrove formation is now well inland, and there are other areas of mangrove that are isolated and dead under too exposed and too saline conditions. In the small bay areas along the north coast *Rhizophora, Laguncularia,* and *Avicennia* comprise the swamps. None of these species has developed extensively nor are the areas of swamp large. Associated with the mangrove

in drier areas are specimens of *Thespesia populnea, Picrodendron macrocarpum, Suriana maritima*, and *Amyris elemifera*. Behind the mangroves may be shallow flats covered now with *Batis, Sesuvium, Salicornia*, and other halophytes. In among the mangroves and elsewhere along the northern coast of Beata are large piles of conch shells. Some of these appear quite old, others more recent. Krieger (l.c.) has mentioned these piles as composed of pure *Strombus gigas*.

The most interesting vegetation on Beata occurred on the central plateau area that Ostenfeld and Wetmore found too difficult to penetrate. Their descriptions of the area, however, are good. The rock surface is almost smooth in places, while in other regions the surface has become badly eroded into sharp edges so characteristic of southern coastal outcrops of limestone on other islands. This is the dogtooth limestone of the Antilles. Ostenfeld noted the peculiarity of this limestone, that is, its metallic ring when struck. Smaller rocks actually ring when hit. The surface of the plateau shows signs of crustal adjustment, with cracks of varying sizes making progress on foot difficult. Crevices eight to ten feet wide were common, and some of these extended approximately sixty feet or more down into the surface. Compression of the island apparently caused piling of large blocks of limestone in other places. Small fractures were evident, and many of these showed additional weathering or erosion due to root action by plants. These smaller cracks had narrow openings but widened downward in contrast to the large crevices, which tapered downward.

The vegetation of this limestone plateau area was complex in its composition (FIG. 5). It is best described as a thorn shrub. The species comprising the vegetation were numerous. While the area seemed barren of flowers or fruit, by careful search the majority of the dominant plants were collected in either flower or fruit and could be identified. One very common tree, assigned to the Leguminosae in the accompanying list, could not be found in fertile condition and remains unidentified. The impression one carries from work on the vegetation of the plateau is that of a thorn-riddled shrub growth. The spiny trunks of *Opuntia moniliformis* were most foreboding in appearance and in the hazard they presented (FIG. 6). Lesser spines occurred on the branches or leaves of *Malpighia setosa, Securinega acidoton, Guettarda stenophylla, G. xanthocarpa, Cryptorhiza haitiensis, Malpighia domingensis, Reynosia cuneifolia, Isodorea leonardii, Randia parvifolia, Hippomane spinosa, Trichilia cuneifolia, Cameraria angustifolia, Caesalpinia anacantha*, and *Cephalocereus polygonus*. In addition the poisonous properties of *Comocladia dodonea, C. mollifolia, Metopium brownei, M. toxiferum, Jatropha hernandifolia* and *Hippomane spinosa* were well known, and these plants, too, had to be avoided.

Nowhere on the island did I see trees of fifty to eighty feet as mentioned in the coastal survey report of 1868. The tallest plants were specimens of *Haitiella ekmanii* which stood out in the profile of the forest canopy. These are the "thrinax-like plants" mentioned by Ostenfeld. The largest trees on the island were *Sarcomphalus domingensis, Coccoloba pubescens, C. diversifolia, Bursera simaruba*, and *Ficus populnea* var. *hispaniolae*. A

single specimen of *Clusea rosea*, also one of the more massive plants on Beata, was seen. Around these few conspicuous plants the vegetation consisted of a dense growth of smaller trees and shrubs, much branched and forming an intertangled canopy of branches six to ten feet above the ground. Many of these plants were microphyllous or had leathery shiny leaves. Broad-leaved plants such as *Ulbrichia beatensis* were few. *Comocladia dodonaea* and *C. mollifolia* appeared to be the most numerous. *Cordia buchii* and *Tabebuia ostenfeldii* in full flower presented the most color. The other common species were *Malpighia setosa* and *M. domingensis*, *Capparis flexuosa*, *Krugiodendron ferreum*, *Schoepfia obovata*, *Randia parvifolia*, *Exostemma caribaeum*, *Elaeodendron xylocarpum* (extremely variable in leaf size and shape), *Plumeria obtusa* (also extremely variable), *Securinega acidoton* and *S. neopeltandra*, *Amyris granulata*, *Isodorea leonardii*, *Trichilia cuneifolia*, *Reynosia cuneifolia*, *Samyda pubescens*, *Guettarda xanthocarpa* and *G. stenophylla*. *Opuntia moniliformis* was the only cactus seen in the shrub thickets. Herbaceous growth was conspicuously lacking throughout the interior of the island. *Setaria setosa* was the only grass found in the woodland. *Euphorbia hepatica* and *Commelina diffusa* occurred on the rocks or in pockets of soil. *Zephyranthes bifolia*, a bulbous plant, occurred in small pockets. This plant was not in flower at the time I visited the island, but transplanted bulbs later flowered at Barahona and in the greenhouse in Cambridge. The absence of herbaceous growth was as conspicuous as the extensive development of vines. Much of the progress through the interior was possible only with the continuous stroking of a machete. *Galactia dictyophylla*, *Mesechites repens*, five species of *Cissus*, *Stegnosperma cubense*, *Paullinia jamaicensis*, *Aristolochia bilobata*, and *Chiococca alba* were rampant. Equally troublesome to the foot traveler was the accumulation of dried leaves on the ground. Apparently decay occurred very slowly, for the leaves were deep in many places, and as they hid not only the rough dogtooth surface of the limestone but also the small depressions and many crevices, progress had to be made cautiously.

Epiphytes in general were few in the thorn shrub area. Tillandsias were relatively abundant in a few places but generally scarce when compared with a comparable location elsewhere in Barahona province. Only two species of orchids were found, and these occurred only on the very low branches or the prostrate trunks, as described by Ostenfeld.

Six and a quarter miles to the south of Beata is the island called Alta Vela, occasionally spelled Alto Vela (FIG. 7). From a botanical point of view this island was less interesting than Beata, but historically Alta Vela is better known and has played an often forgotten role in American history.

Alta Vela Island was discovered by Columbus on August 20, 1494. As J. B. Thacher (*Christopher Columbus* 2: 336–7. 1903) describes the event, Columbus "anchored at a small island, or rather a rock which rises singly out of the sea opposite to the long cape stretching southward from the center of the island, to which promontory he gave the name of Cape Beata.

The rock at which he anchored had an appearance at a distance of a tall ship under sail and he called it Alta Vela. Several seamen were ordered to climb the rock to watch out for the two lost ships of his squadron. Descending from the summit the sailors killed on the island of Alta Vela eight sea wolves which were sleeping on the sands, knocked down many pigeons and other birds with sticks and took others with their hands; for in this unfrequented island the animals seemed to have none of the wildness and timidity which is produced by hostility of man."

As Alta Vela, like Beata, was destitute of natural water supplies, few ships stopped there except for food or firewood, and no evidence can be found in the literature that the island was ever occupied by other than transients. It is a small island about three quarters of a mile long and a half-mile wide composed originally of a "remarkable bell-shaped hill, the summit 500 feet above sea level." Alta Vela Island almost became a possession of the United States in the middle of the nineteenth century, and the dispute over the ownership of it forms an interesting part of American political history. The story is told in detail in the Senate Executive Document No. 38 of the second session of the Fortieth Congress (1868), in Document No. 17 of the third session of the Forty-first Congress (1871), and reviewed in "The United States and Santo Domingo 1798–1873," pages 287–337, by Charles C. Tansill. I am quoting liberally from these documents in summarizing the history of Alta Vela.

The United States Congress, on Aug. 18, 1856, passed a law to legalize claims of American citizens to unoccupied islands with guano deposits. The law stated, "When any American citizen chances to discover a deposit of guano on any island, rock or key not within the lawful jurisdiction of any other government and takes peaceful possession thereof, and occupies the same, such island, rock or key may, at the discretion of the President, be considered as appertaining to the United States." As soon as practicable the claimant should give notice to the Department of State of such discovery and occupation describing the island, rock or key and the latitude and longitude thereof as near as may be, and showing that such possession was taken in the name of the United States and should furnish satisfactory evidence to the State Department that such island was not at the time of discovery thereof or the taking possession of and occupation thereof by the claimant in the possession or occupation of any other government. After these conditions were met the discoverer might at the pleasure of Congress be allowed "exclusive rights of occupying such island, rocks or keys for the purpose of obtaining guano and of selling and delivering the same to citizens of the United States."

It was with this law in mind that a schooner owned by the Baltimore firm of Patterson and Murguiendo took possession of Alta Vela on Feb. 23, 1860. In May and June the Secretary of State and the State Department of Buchanan's administration were notified that a claim to the uninhabited and unclaimed island was made for the United States. Mining operations were carried on, and when a Dominican vessel stopped at the island in September of that year, its officers were given a sample of the guano. In

early October the Dominican government ordered the Americans to leave the island, which they refused to do, and on the twenty-third of October a vessel of the Dominican government landed troops who arrested the workers and removed the mining equipment. These workers and a company representative sent to obtain their release were held prisoners in Santo Domingo city for nearly thirty days, in spite of promises of safe conduct. The company protested the action to the American Secretary of State. Buchanan's term expired, Johnson became president, and Seward was appointed Secretary of State. Black, the former Secretary, was hired as counsel for the protesting firm. Seward at the time was negotiating with the Dominican government for the use of Samana Bay at the northeast corner of Hispaniola as a United States naval base and was not anxious to force the claim of the previous administration regarding Alta Vela. In fact Seward was thought to be guilty of duplicity when he informed a competing firm, Webster & Co. of New York, that in his opinion the territory under question belonged to the Dominican government. An intimate friend of Seward was found to have a large interest in Webster & Co., and the rage against Seward and Johnson increased when it was learned that the Dominican government had given permission to the Webster company to remove guano from Alta Vela. Patterson and Murguiendo Co., the original claimant to the Alta Vela guano, reported to the State Department that at the current rate of mining and exporting at two thousand tons per month, the guano would soon be exhausted. When the House of Representatives, a few months later on Feb. 24, 1868, agreed on a resolution of impeachment against Johnson, the events which had occurred in Seward's handling of the Alta Vela dispute ranked high. The House Committee on Foreign Affairs, which investigated the dispute, reported, "We are compelled to say that the case is too clear to allow of the least hesitation. Santo Domingo was guilty of an inexcusable outrage upon the rights of the memorialists and a gross insult to the United States." In May of 1869 an appeal was made to Hamilton Fish, the new secretary of state under Grant, for a decision in the case. Finally in May of 1870 Fish reported to the attorneys for Patterson and Murguiendo that all of the documents and pertinent correspondence had been placed before President Grant, who after due consideration determined that no sufficient reason appeared for reconsidering the conclusions heretofore reached.

With that decision Alta Vela dropped from the news. Apparently in due time the guano deposits were mined out. From information and estimates which appeared in the Senate Executive Documents, Alta Vela once contained fifteen thousand tons of guano. An analysis of this material showed it contained 29.16% phosphoric acid and 70.84% lime.

Apparently the mining operation forced the sea birds to change their habits, for no large numbers of them roost on Alta Vela today. The scars of the mining operations on the leeward slopes of the island are still visible, and artifacts of mining still litter portions of the hill (FIG. 10).

Alta Vela, with no wood, water, or fertile soil, remains a desolate outpost. Today a single house and a few small supply buildings take care of the

needs of a lighthouse keeper. The lighthouse at the summit of the hill represents one of the southernmost beacons of the Greater Antilles (FIG. 9).

For the botanist visiting Alta Vela today there is little of interest. The island is bounded by severe wave-washed and undercut cliffs (FIG. 8).

Around the island the bottom drops off sharply, and within a short distance of the shore it consists primarily of boulders offering poor if any anchorage in the heavy seas that continuously wash the island. Landing from the destroyer escort which provided transportation for me proved hazardous, and in fact the vessel stayed under power to hold its position during my visit to the island. Apparently previous occupants of the lighthouse keeper's position tried to grow vegetables and crops of cotton on the slopes on the leeward side. The current keeper informed me he had learned this to be futile, for the lack of sufficient moisture and the frequent dousings with salt spray killed off all his attempted crops.

Alta Vela today has primarily a weedy vegetation. The few woody plants which occurred on the island were located in ravines, primarily on the leeward side of the island (Fig. 10). Those shrubs struggling for existence on the crest of the hill or on the windward side were severely wind-blown and malformed (FIG. 9). These woody plants were few. *Capparis flexuosa* and *C. cynophallophora*, *Ficus populnea*, *Pithecellobium unguis-cati* and *Duranta repens* represented the largest woody plants. *Morinda royoc*, *Iresine angustifolia*, *Eupatorium corymbosum*, and *Sesbania sericea* were the most common plants of low stature. The coastal or flatland areas were dominated by weedy species. In fact the list of twenty species found on Alta Vela and not on Beata would show only pan-Caribbean, if not pan-tropical weeds. There are no evidences of even interesting species of limited range found on the island.

Many of the areas, particularly the mined-out areas on Alta Vela, appeared from the sea to be grassy meadows (FIG. 10.). These were deceptive even from nearer by. The grasses and sedges giving this impression, *Paspalum bakeri*, *P. adspersum*, *Setaria geniculata*, *Cyperus compressus*, *Fimbristylis spathacea*, formed dense stands one to three feet high. However, mixed in with these grasses and hidden by them were extensive matlike growths of *Opuntia antillana*. *Opuntia dillenii* was common around the edges of these swales. On the exposed rocky outcrops at the sea-cliff margin of the island the vegetation is stunted and consists of the usual halophytes of such areas. *Lithophila muscoides*, *Portulaca oleracea*, *Talinum paniculatum*, *Iresine angustifolia*, and *Sesuvium portulacastrum*. Common in such locations, however, is the turk's cap cactus, *Cactus lemari*.

Alta Vela, by contrast to Beata, has a flora consisting entirely of a woody pauperous vegetation. Whether the island ever had the interesting flora found on Beata is impossible to speculate. Probably its environment was such that shelter was lacking, and only the most hardy of Caribbean plants could grow. Mining operations may have removed some of the vegetation from the sheltered leeward slopes, but the abundance of birds

necessary to produce the guano may also have played a role in the development of the present weedy vegetation.

The vegetation on Alta Vela offers little information regarding the affinities of the flora of the southern coast of Hispaniola, particularly when contrasted with that of Beata.

Beata Island possesses a flora consisting of 118 genera and 168 species, as based on the collection records of Ostenfeld and Howard. The vast majority of the species on Beata are wide-ranging species, but thirty-five of them, representing twenty per cent of the flora, are species of limited distribution and contribute information regarding the affinities of this islet. In addition, three good species are to be currently regarded as endemic to Beata. These are *Ulbrichia beatensis*, *Tabebuia ostenfeldii*, and *Galactia dictyophylla*. One species, *Euphorbia hepatica*, is known elsewhere only from Navassa Island. *Guettarda xanthocarpa*, *G. stenophylla*, *Amyris granulata*, and the hybrid between *Coccoloba uvifera* and *C. pubescens* are known only from the vicinity of Trujin on the Barahona peninsula. *Salicornia bigelovii* has also been collected by Ekman on Gonave Island but is not known from the main island of Hispaniola. Two species, *Elaeodendron ehrenbergii* and *Antirrhoea elliptica*, have been previously collected only in the Cul de Sac, with the latter known only from Isla de Cabritos in Lake Enriquillo.

In general the relationship of the vegetation of Beata Island is with the dry-land shrub vegetation extending from southeastern Haiti through the Barahona peninsula into the Enriquillo valley and eastward to the vicinity of Azua. There is no indication in the Beata flora of affinities with the vegetation of the western end of the southern peninsula of Haiti, a floristically distinct area frequently pointed out by Ekman. Geologically, as well as botanically, Beata appears to be a fragment of recent fracture from the Barahona peninsula.

A comparison of Beata Island with the other islands intensively studied by Ekman reveals that for its small size and lack of topography Beata has indeed a very interesting vegetation. Beata ranks in size with Navassa (*Arkiv för Botanik* **22A** (16): 1–12. 1929). The number of species found on Beata is greater, the percentage of endemics about equal; the percentage of introduced or weedy species is much less and the number of interesting species of limited range much higher. The larger size of Gonave Island and the greater range of topography, coupled with its location, have produced a much larger flora, 928 species, than that found on Beata (*Arkiv för Botanik* **23A** (6): 1–73. 1930). The same is true of Tortue Island, which has 889 species (*Arkiv för Botanik* **22A** (9): 1–61. 1929). Gonave, Tortue, and Navassa are reported to have a soil developed in all or parts of the islands. All seem to receive sufficient rainfall to support the diversified vegetation present. All are in the paths of prevailing winds and currents which would aid the introduction of additional species of plants from the mainland of Hispaniola. Beata stands by contrast. No accurate rainfall records are available for Beata. According to the naval personnel stationed on the island the rainfall is very slight. The catchments estab-

lished on the buildings on Beata do not provide sufficient rainfall for the personnel, and water must be imported. The accumulation of leaves on the surface of the plateau supports the conclusion that sufficient moisture is not available for decay of vegetation. Interestingly, termite action was not noticed on the island. The lack of soil on Beata has prevented the development of a fern flora or of extensive herbaceous growth. The location of Beata, south of the Barahona peninsula, has prevented the introduction of many species from the mainland of Hispaniola by natural transport. Particularly striking in the Beata flora was the complete lack of certain major groups of plants, such as the ferns, and the few examples of epiphytes and palms seen. Families and genera of flowering plants expected in similar dry thorn shrub areas of Hispaniola were also lacking. In particular the absence of members of the Piperaceae, Loranthaceae, Flacourtiaceae, Myrsinaceae, Sapotaceae, Labiatae, Solanaceae, Rutaceae, and Acanthaceae was noted. Characteristic genera such as *Pilea*, *Zanthoxylum*, *Trema*, *Phyllostylon*, *Maytenus*, *Casearia*, *Sideroxylon*, *Bumelia*, *Acacia*, and *Mimosa* were missing from the flora. Additional work on the flora or Beata may be expected to produce additional distributional records, but such work must be accompanied by an examination of the vegetation from Lago Trujin to Punta Beata for comparison. The succession of plant life in the abandoned salt pans should be followed for information on replacement of vegetation in dry-land areas. Nowhere could a study of the development of such a high percentage of toxic and spiny plants as related to the factors of their environment be better attempted.

A LIST OF PLANTS OF BEATA AND ALTA VELA ISLANDS

HYDROCHARITACEAE

Thalassia testudinum Koenig. Beata; *Ostenfeld s.n.*, Howard by observation. — A marine plant particularly common off the north shore in shallow water.

GRAMINEAE

Cenchrus brownii R. & S. Alta Vela; *Howard 12474.*

Cenchrus myosuroides H.B.K. Alta Vela; *Howard 12464.* — Found near the crest of the hill. The stems prostrate, to six feet long.

Cenchrus pauciflorus Benth. Beata; *Howard 12419.* — Plant of open sandy area at the northwest corner of the island. Stems prostrate, radiating.

Panicum adspermum Trin. Alta Vela; *Howard 12462.* — A prostrate plant with radiating branches.

Panicum maximum Jacq. Beata; Ostenfeld by observation, *Howard 12389.* — A common grass in areas around bays and old salt pans.

Paspalum bakeri Hackel. Alta Vela; *Howard 12480.* — A common clump grass, perhaps the most abundant and dominant plant on old

guano mined areas. Previously collected on Inagua, Cuba and Barbuda. This is the first record from Hispaniola.

Setaria geniculata (Lam.) Beauv. Alta Vela; *Howard 12471.*

Setaria setosa (Sw.) Beauv. Beata; *Howard 12493.* — Perhaps the only grass growing on limestone in brush thickets. Plants few, scattered.

Sporobolus domingensis (Trin.) Kunth. Beata; *Ostenfeld 330*, Howard by observation. A beach grass dominating sandy areas in the northeast corner of the island. Sterile in August.

Sporobolus pyramidatus (Lam.) Hitch. Alta Vela; *Howard 12460.* — A rare plant; only one specimen seen on the lee side of the island.

CYPERACEAE

Cyperus compressus L. Alta Vela; *Howard 12465.* — An uncommon sedge found on rocks near the lighthouse.

Cyperus nanus Willd. Beata; *Howard 12400.* A single specimen found on the limestone benches near the coast.

Cyperus planifolius L. C. Rich. Beata; *Howard 12447.* Alta Vela; *Howard 12467.* A clump-forming plant found on sand, especially in swales on Beata. On Alta Vela this species is more common, being found in large colonies on the leeward slopes where guano had been mined. This area has been burned frequently, and the charred bases of these plants give the hill a blackened appearance from the sea.

Fimbristylis spathacea Roth. Alta Vela; *Howard 12466.* — A sedge occurring from sea level to the crest of the island. More abundant at the lower elevations.

Mariscus bruneus (Sw.) Clarke. Beata; *Ostenfeld 318, 339.*

Remirea maritima Aubl. Beata; *Howard 12427.* — This unusual sedge was particularly common in the grassy areas in the northeast corner of Beata Island on sand at the edge of limestone. The white bracts cause the plant to be conspicuous even from a distance. The species has been reported previously only from the vicinity of Barahona on Hispaniola. One locality record is known from Puerto Rico. The species is more common in Trinidad, South America, and Africa.

BROMELIACEAE

Tillandsia balbisiana Schl. Beata; *Howard 12349.* — An epiphyte in the shrub thickets.

Tillandsia circinnata Schl. Beata; *Ostenfeld 327.*

Tillandsia recurvata L. Beata; *Howard 12508.* — A common epiphyte found only on the western side of the island on shrubs in the lee of the cliff face.

Tillandsia usneoides L. Beata; *Ostenfeld 315, Howard 12380.* An epiphyte of general occurrence.

Tillandsia utriculata L. Beata; *Howard 12374.* — The largest epiphyte found in the interior of the island.

COMMELINACEAE

Commelina diffusa Burm. f. Beata; *Howard 12494.* — A fleshy herb found on the limestone in the shrub area away from the coast.

PALMAE

Haitiella ekmanii (Burret) Bailey. Beata; *Howard 12369, 12420.* — Apparently the only palm on Beata Island. At the northern end of the island this species is quite abundant in windswept shrub formations, averaging here ten feet in height. Toward the southern end and in the interior in the shrub thickets the palm reached forty feet in height. This species was known previously only from the southern peninsula of Haiti at Anses-à-Pitre on Morne Savane Lafleur (Contrib. Gray Herb. **165**: 5–9, 1947).

AMARYLLIDACEAE

Zephyranthes bifolia (Aubl.) Roem. Beata; *Howard 12499-a.* — A bulbous plant occurring in cracks and crevices in the limestone in the interior of Beata. The plant was in sterile condition in August when collected. The bulbs collected were planted and flowered in November in Barahona, while others flowered in March in Cambridge, Mass., allowing positive determination. The species is relatively common on limestone in Hispaniola but is not known elsewhere to me at such a low altitude.

ORCHIDACEAE

Laeliopsis domingensis Lindl. (*Broughtonia domingensis* Rolfe). Beata; *Ostenfeld s.n., Howard 12402, 12428.* — An epiphyte of the shrub area, widespread on the island. Ostenfeld notes that these plants occur primarily on the lower branches or trunks of the shrubs. I can verify that this seems to be the pattern of occurrence of the species on the island.

Oncidium intermedium Bert. Beata; *Howard 12518.* — Locally abundant in the thorn shrub areas along the western coast of the island.

ULMACEAE

Celtis trinervia Lam. Beata; *Howard 12372.* — Trees of fifteen feet with green fruit. In the thorn shrub areas on limestone.

MORACEAE

Ficus populnea Willd. var. **hispaniolae** Urb. Beata; *Howard 12516.* Alta Vela; *Howard 12482.* — A twenty-foot tree on Beata Island occurring in the thorn shrub. The striking dark green foliage makes

this plant conspicuous in the thickets. On Alta Vela this species is generally found on the windswept eastern slopes, where plants from one to fifteen feet tall were seen.

URTICACEAE

Rousselia humilis (Sw.) Urb. Beata; *Howard 12375.* — One of the few terrestrial herbs in the limestone areas.

OLACACEAE

Schoepfia obovata Wr. Beata; *Howard 12383.* — An exceedingly common shrub of thorn thickets. Plants occur to eight feet tall.

ARISTOLOCHIACEAE

Aristolochia bilobata L. Beata; *Howard 12366.* — A sterile vine with distinctive foliage.

POLYGONACEAE

Coccoloba diversifolia Jacq. Beata; *Howard 12361.* — A common tree fifteen to twenty-five feet in height in thickets on limestone.

Coccoloba pubescens L. Beata; *Howard 12352.* — One of the largest trees on Beata Island reaching twenty-five feet in height. It occurs on the limestone from the benches of the west coast to the center of the island.

Coccoloba subcordata (DC.) Lind. Beata; *Howard 12488.* — Prostrate or ascending shrubs occurring on the limestone plateau.

Coccoloba uvifera L. Beata; *Howard 12489.* Alta Vela; *Howard 12453.* — Trees reaching fifteen feet are found along the coast in sandy areas.

Coccoloba uvifera × C. pubescens. Beata; *Howard 12499.* — This new hybrid was first discovered south of El Caiman on Barahona peninsula, where several stands of the plant had developed between colonies of the parents. The hybrid is a true intermediate with the leaf shape of *C. uvifera* but the venation and texture of *C. pubescens.* The fruits were all sterile but of the size and shape of those of *C. uvifera.* Only a single specimen, eight feet tall, was found on Beata at the southern end of the sandy strip which occurs on the northwest corner of the island.

CHENOPODIACEAE

Atriplex pentandra (Jacq.) Standl. Beata; *Howard 12451.* — A single plant was found on the rocks of the coastal bench. Moscoso lists this plant without specific location in his catalogue of plants from the island. This is the first specimen of this species I have collected in Hispaniola.

Salicornia bigelovii Torr. Beata; *Howard 12442.* — A relatively abundant species mixed with *Batis* and *Sesuvium* along the lagoon

near the salt pans. Moscoso does not record this species from Hispaniola although Ekman collected it on Gonave Island near Trois Louis. Ekman reported the species was not found on the mainland, although it is found on the Atlantic coast of the United States and in Cuba. This collection represents the second record from Hispaniola, but again from an island, not the mainland.

AMARANTACEAE

Amaranthus gracilis Desf. Alta Vela; *Howard 12458.* — This species occurred as a weed in an old field on Alta Vela. Moscoso does not list it in his Catalogue, although I have now collected the taxon at Manzanilla and in the Enriquillo basin. Ekman found the same species on Gonave.

Celosia nitida Vahl. Beata; *Ostenfeld 320.*

Iresine angustifolia Euph. Alta Vela; *Howard 12456.* — The species occurs as a weed on the area disturbed by guano mining.

Lithophila muscoides Sw. Beata: *Ostenfeld 345*, *Howard 12426.* Alta Vela, *Howard 12461.* — On both Beata and Alta Vela this species was a prostrate plant of limestone coastal benches.

NYCTAGINACEAE

Boerhaavia caribaea Jacq. Beata; *Howard 12487.* Alta Vela; *Howard 12472.* — A sporadic weed occurring primarily in the grassy areas.

Neea subcoccinea Heimerl. Beata; *Howard 12379.* — Characteristic shrub of limestone plateau. Plants to eight feet tall with widespreading branches. This species is apparently endemic to Barahona province and has been recorded previously only from that vicinity.

BATIDACEAE

Batis maritima L. Beata; Howard by observation. — A common woody perennial along the lagoon and edges of the salt pans on Beata.

PHYTOLACCACEAE

Rivina humilis L. Alta Vela; *Howard 12476.* — Plants to two feet tall were relatively common in the sheltered areas on Alta Vela.

Stegnosperma cubense A. Rich. (*Stegnosperma halimifolium* auct.). Beata; *Ostenfeld 319*, *Howard 12507.* — A trailing or scrambling fleshy-stemmed to woody bush quite common at the edge of the sand and limestone on the western coast of Beata Island. Moscoso does not give a specific location for the occurrence of this species in Hispaniola. D. J. Rogers (Ann. Mo. Bot. Gard. 36: 475. 1949) cites two collections by *Fairchild*, numbers *2605* and *2606*, from Beata Island. He likewise records collections by Ekman from Massif des Cahos, Barahona, and Sierra de Ocoa.

AIZOACEAE

Sesuvium portulacastrum L. Beata; Ostenfeld and Howard by observation. — A common fleshy herb on sandy areas and occasionally on the limestone coastal benches.

PORTULACACEAE

Portulaca oleracea L. Beata; *Ostenfeld 344, Howard 12450.* — A common weed around the buildings in the northwest corner of Beata.

Portulaca phaeosperma Urb. Beata; *Ostenfeld 346, Howard 12405.* — A fleshy herb of sand and of limestone coastal benches.

Talinum paniculatum (Jacq.) Gaertn. Alta Vela; *Howard 12477.* — A stout herb of hillsides on both windward and leeward sides.

ANNONACEAE

Annona bicolor Urb. Beata; *Howard 12356.* — A shrub of the limestone plateau. Plants to fifteen feet tall with attractive reddish purple flowers. This species had previously been reported only from the vicinity of Barahona.

LAURACEAE

Cassytha americana Nees. Beata; *Howard 12417.* — An orange-colored parasitic vine on shrubs of the coastal limestone benches.

CRUCIFERAE

Cakile lanceolata (Willd.) Schulz. Beata; *Howard 12444.* — A fleshy herb of sandy areas at the northwest corner of Beata.

CAPPARIDACEAE

Capparis cynophallophora L. Beata; *Ostenfeld 309, Howard 12406.* Alta Vela; *Howard 12485.* — This species occurs in the shrub area on the limestone plateau on Beata. It is also the largest and most common tree on the leeward slopes of Alta Vela.

Capparis flexuosa L. Beata; *Ostenfeld 317, Howard 12504, 12393.* Alta Vela; *Howard 12483.* — A common scrambling shrub of the thickets of Beata and on the leeward slopes of Alta Vela.

LEGUMINOSAE

Caesalpinia anacantha Urb. Beata; *Howard 12432.* — One of the common shrubs in the windswept area on limestone at the northern end of Beata. Ekman found this species on Gonave Island and it has been collected at Montagnes du Trou d'Eau in Morne a Cabrits.

Caesalpinia ciliata (Berg.) Urb. Beata; *Ostenfeld 325.* — Previously recorded from Haiti only.

Caesalpinia glandulosa Bert. Beata; *Howard 12435.* — A spreading shrub to five feet tall in the windswept shrub area on limestone at the northern end of Beata. Moscoso reports that this species was collected by Bertero in the Dominican Republic, but the location is unknown.

Canavalia maritima (Aubl.) Thou. Beata; *Howard 12392.* — A common beach vine on sandy areas of Beata.

Cassia buchii Urb. Beata; *Ostenfeld 313, Howard 12448.* — This low shrub was found only on limestone. Urban, in determining the plants collected by Ostenfeld, felt that the Beata plants represented a distinct form. I feel this is an ecological variant due to the habitat.

Cassia strigillosa Benth. Beata; *Howard 12399.* — A woody herb of the sandy areas.

Desmanthus virgatus (L.) Willd. Beata; *Howard 12433.* — A woody herb becoming five feet tall. In thicket and windswept areas of the plateau.

Desmodium triflorum DC. Alta Vela; *Howard 12475.* — A prostrate herb occurring on rocks at the summit of the island.

Galactia dictyophylla Urb. Beata; *Ostenfeld 329, 332, Howard 12500.* — This relatively common vine occurs on the limestone cliff faces. The species is endemic to Beata Island.

Indigofera suffruticosa Mill. Beata; *Howard 12398.* — A weedy herb of sandy areas, growing to four feet in height.

Pithecellobium unguis-cati (L.) Benth. Alta Vela; *Howard 12455.* — A low shrub on the windward slopes of Alta Vela.

Sesbania sericea (Willd.) DC. Alta Vela; *Howard 12457.* — A shrub of three feet on the leeward slopes of the island.

Tephrosia cinerea (L.) Pers. Beata; *Howard 12423.* — A common woody herb of sandy beaches.

One of the common small trees of Beata Island remains unidentified (*Howard 12384*). This fifteen-foot tree was not in flower or fruit during my visit there. The leaves are evenly pinnate and consist of four to six leaflets. The leaflets are orbicular-rhombic in outline with the midrib placed off the median. The texture of the leaflets is coriaceous and when dry both surfaces are densely reticulate. In appearance the specimens collected resemble *Pithecellobium*, but until fertile material is available the plant remains unidentified.

ERYTHROXYLACEAE

Erythroxylon areolatum L. Beata; *Ostenfeld 308, Howard 12360.* — A ten-foot spreading shrub relatively common in limestone plateau thickets.

ZYGOPHYLLACEAE

Guaiacum sanctum L. Beata; *Ostenfeld 304, 305, Howard 12502.* — Trees of twenty feet in thickness on limestone.

Tribulus cistoides L. Beata; *Ostenfeld 326.*

RUTACEAE

Amyris elemifera L. Beata; *Howard 12401.* — A shrub of twelve feet occurring on the limestone plateau.

Amyris granulata Urb. Beata; *Howard 12344.* — A relatively common shrub of twelve feet occurring on the limestone at all elevations. The species was sterile in August. The type, from Trujin, was collected by Abbott.

SIMARUBACEAE

Picrodendron macrocarpum (A. Rich.) Britton. Beata; *Ostenfeld 293, Howard 12430.* — A common tree around the old salt pans. Plants reaching fifteen feet with spreading branches.

Suriana maritima L. Beata; Howard, by observation. — A common shrub of the sandy beaches on the west coast.

BURSERACEAE

Bursera simaruba (L.) Sarg. Beata; *Ostenfeld 253, 322, Howard 12515.* — Due to its colored bark, this is the most conspicuous tree in the open thorn thickets on limestone.

MELIACEAE

Trichilia cuneifolia (L.) Urb. Beata; *Howard 12388.* — A shrub of twelve feet occurring on limestone. Previously reported from Azua to Barahona on Hispaniola.

MALPIGHIACEAE

Malpighia domingensis Small. Beata; *Howard 12346.*

Malpighia setosa Spreng. Beata; *Howard 12501.*

Stigmaphyllum lingulatum (Poir.) Small var. **sericans** Ndz. Beata; *Ostenfeld 335,* Howard by observation.

EUPHORBIACEAE

Croton lucidus L. Beata; *Howard 12440.* — A four-foot shrub common along the edges of the old salt pans.

Croton polytomus Urb. Beata; *Howard 12407.* — Previously reported from Cadets on the northern peninsula of Haiti and considered endemic there. This was a common seven-foot shrub in the thickets.

Euphorbia buxifolia Lam. Beata; *Howard 12445.* — An herb of sandy and grassy areas and less frequent on limestone benches.

Euphorbia hepatica Urb. Beata; *Howard 12425.* — A prostrate plant growing in small holes in the limestone. This species was based on material collected by Ekman on Navassa Island. This is the second collection recorded for this insular endemic.

Gymnanthes lucida Sw. Beata; *Howard 12505.* — A common shrub in thickets, to eight feet tall.

Hippomane manchinella L. Beata; Howard by observation. — Common in restricted areas of coastal sand.

Hippomane spinosa L. Beata; *Howard 12385.* — A shrub of eight feet to a tree of twenty feet. Common in thickets and thorn shrub on the plateau. This species has been previously collected in Haiti. The toxicity of the sap of this plant far exceeds any of the other dermatitis-producing plants that I have encountered in the Antilles. After collecting specimens from one of the plants seen on Beata, I accidentally ran the sharpened branch through the shoulder of my shirt, without breaking the skin. A few hours later my shoulder became painful, and I discovered that a large blister was developing. Liquid continued to accumulate, forming several blisters, and the largest, by the time it broke, was fully two inches in diameter and an inch high. The amount of liquid that accumulated in the four large blisters produced was copious. A light compress was required for several days to protect the affected area, but no unusual soreness or after-effect was experienced. The sailors who are stationed at Beata Island confirmed the unusual blistering properties of this species and recognized them as being far more severe than those of *Hippomane manchinella.*

Jatropha hernandifolia Vent. Beata; *Howard 12351.* — A common weak-structured shrub to eight feet tall. The flowers are white and the mature fruit bright green.

Jatropha multifida L. Beata; *Howard 12376.* — It is difficult to believe that this plant, which occurs occasionally in thorn shrub areas of Barahona peninsula and in the plateau area of Beata, is not a native of the region.

Securinega acidoton (L.) Fawc. & Rend. Beata; *Howard 12503.* — One of the common very spiny shrubs of the thorn shrub thickets on the plateau.

Securinega neopeltandra (Griseb.) Urb. (*Chascotheca domingensis* Urb.). Beata; *Howard 12358.* Alta Vela; *Howard 12481.* — A common low shrub on both Beata and Alta Vela, which was collected in fruit in August.

ANACARDIACEAE

Comocladia dodonaea (L.) Urb. Beata; *Ostenfeld 331, Howard 12514.*

Comocladia mollifolia Ekm. & Helw. Beata; *Howard 12517.* — Barkley, who has studied my collections of *Comocladia*, refers this material to

C. mollifolia, which is based on an Ekman collection from Las Villas province in Cuba. I have previously collected this species from the vicinity of Pedernales. Together these two species of *Comocladia*, represented by spindly shrubs with a characteristic crown of pinnate leaves, represent the most abundant plants in most areas of the limestone plateau.

Metopium brownei (Jacq.) Urb. Beata; *Ostenfeld 252, 321, Howard 12519.*

Metopium toxiferum (L.) Krug & Urb. Beata; Howard by observation. — Both species of *Metopium* were observed growing to the rear of the sandy beach areas on the west coast. Both were present, but less abundant, on the limestone plateau.

CELASTRACEAE

Elaeodendron ehrenbergii Urb. Beata; *Ostenfeld 251, 298, 299, 301, Howard 12396.* — One of the most common plants in the more fertile sheltered areas back of the sand and coastal benches on the west coast. The plants averaged fifteen feet in height and were colorful with the abundant yellowish fruits.

Elaeodendron xylocarpum (Vent.) DC. Beata; *Howard 12490.* — A six-foot shrub on the limestone plateau. Previously known only from the vicinity of Barahona in Hispaniola but of widespread occurrence in the Antilles.

Maytenus reynosioides Urb. Beata; *Fairchild 2613.* — A microphyllous shrub apparently on the limestone.

Rhacoma crossopetalum L. Beata; *Howard 12434.* — A common shrub on limestone, to seven feet tall.

Schaefferia frutescens Jacq. Beata; *Howard 12373.* — A common shrub to ten feet tall, with red fruit.

SAPINDACEAE

Cardiospermum corindum L. Alta Vela; *Howard 12486.* — A weedy plant on open hillsides.

Paullinia jamaicensis Macf. Beata; *Howard 12363.* — An abundant but localized vine on the plateau. This appears to be the first record of the species from Hispaniola. It has been recorded previously from Jamaica and Cuba.

Sapindus saponaria L. Beata; *Ostenfeld 307, Howard 12397.* — A coastal tree of sandy beach areas, to twenty feet tall.

RHAMNACEAE

Colubrina ferruginea Brong. Beata; *Howard 12390.* — A coastal shrub often found with *Rhizophora* and *Thespesia* at the north end of Beata.

Colubrina reclinata (L'Her.) Brong. Beata; *Howard 12496.* — A common low shrub of sandy areas along the west coast.

Krugiodendron ferreum (Vahl.) Urb. Beata; *Howard 12342.* — A fifteen-foot shrub on the limestone plateau area.

Reynosia cuneifolia Urb. & Ekm. Beata; *Howard 12353.* — Abundant in thorn shrub on cliff faces and on the plateau.

Sarcomphalus domingensis (Spreng.) Krug & Urb. Beata; *Ostenfeld 320, Howard 12348.* Generally a small tree averaging fifteen feet, but with a few very old and large specimens in the dense thickets on the plateau. One of the largest trees on Beata.

VITACEAE

Cissus caustica Tussac. Beata; *Howard 12341.*

Cissus fuertesii Urb. Beata; *Howard 12359.*

Cissus macilenta (Planch.) Urb. Beata; *Ostenfeld 336.*

Cissus micrantha Poir. Beata; *Howard 12362.*

Cissus trifoliata L. Beata; *Ostenfeld 311, Howard 12513.* — These species of *Cissus* were equally abundant all over the island. In many places a single species or combination of them made penetration through the thorn shrub a matter of cutting one's way with a machete.

TILIACEAE

Corchorus hirsutus L. Beata; *Howard 12404.* — A shrub of sandy areas, to four feet high.

MALVACEAE

Gossypium hirsutum L. var. **punctatum** (Schum.) Hutch. Beata; *Ostenfeld 324, Howard 12438.* — A few scattered plants on the northwest corner of Beata led Ostenfeld to conclude that agriculture had been conducted there. Around the salt ponds and in the thorn shrub on the eastern coast of the island these plants are even more abundant, without accompanying evidences of cultivation.

Sida acuta Burm. Alta Vela; *Howard 12470.* — Woody herb around the lighthouse.

Thespesia populnea (L.) Soland. Beata; *Howard 12410.* — Low to almost prostrate coastal tree near the mangroves.

Ulbrichia beatensis Urb. (*Armouria beata* Lewton). Beata; *Ostenfeld 312, Howard 12371.* — This species was originally described by Urban from the Ostenfeld collection. The plant was again collected by D. Fairchild and P. H. Dorsett, members of the Allison V. Armour expedition, in 1932 and described by Lewton as a new genus. Both Howard (Bull. Torr. Club **76**: 89–100. 1949) and Kearney (Amer. Midl. Nat. **46**: 111. 1951) have discussed this genus. The collection I made in August was sterile, but the species is easily recognized.

STERCULIACEAE

Melochia tomentosa L. Beata; *Howard 12498.* — A common three-foot shrub on limestone, especially in the windswept northern area.

Waltheria americana L. Beata; *Ostenfeld 314.*

GUTTIFERAE

Clusia rosea Jacq. Beata; *Ostenfeld 296, Howard 12364.* — A single specimen, a tree of twenty feet, was found on the plateau area.

CANELLACEAE

Canella alba Murr. Beata; *Howard 12436.* — One of the largest trees in the thorn shrub around the salt pans. Plants to fifteen feet tall.

FLACOURTIACEAE

Samyda pubescens L. Beata; *Howard 12365.* — A low shrub of six feet on the limestone. A very attractive shrub when in full flower.

TURNERACEAE

Turnera ulmifolia L. Alta Vela; *Howard 12469.* — A common woody herb with brilliant yellow flowers. Abundant in disturbed areas.

PASSIFLORACEAE

Passiflora suberosa L. Beata; *Howard 12449.* — A fleshy vine on sandy areas and coastal benches.

CACTACEAE

Cactus lemari (Monv.) Brit. & Rose (*Melocactus*). Alta Vela; *Howard 12459.* — An extremely abundant turk's head cactus found on the exposed windward slopes and the south end of Alta Vela. Fruit a characteristic bright pink color.

Cephalocereus polygonus (Lam.) Brit. & Rose. Beata; *Howard 12492.* — A strict or candelabra-branched cactus reaching twenty feet in height. Abundant in open area on the limestone. The fruit is a bright red color.

Opuntia antillana Brit. & Rose. Beata; *Howard 12408.* Alta Vela; *Howard 12468.* — The most common low pad-type cactus on both Beata and Alta Vela. In the guano mined areas of Alta Vela this cactus is extremely abundant, although completely hidden by stands of grass and sedge. It is this cactus that makes progress through the "grassy areas" of both islands treacherous. It was observed that the stem segments of *Opuntia antillana* detach and attach themselves to trousers much more readily than those of *O. dillenii.*

Opuntia caribaea Brit. & Rose. Beata; Howard by observation. — This cactus with cylindrical joints was common in many areas of the thorn shrub on the limestone in the shrub thickets of Beata. This is the nastiest of all the cacti in Hispaniola, as the joints detach very readily, and any movement of the plant literally jettisons the spiny segments in all directions.

Opuntia dillenii (Ker.-Gawl.) Haw. Beata and Alta Vela; Howard by observation. — This plant is restricted to coastal areas and to areas of former human occupation.

Opuntia moniliformis (L.) Haw. Beata; *Howard 12387*. — An extremely common tree-type cactus found in the shrub thickets on the limestone plateau. The trunk of this cactus may be six inches in diameter and is covered with spines two to four inches long. The first branch may occur four to six feet from the ground. The flattened branches develop in all directions, and a thick stand of these plants is almost impossible to penetrate. The small flowers are attractive with the outer perianth parts red and the inner bright orange.

RHIZOPHORACEAE

Rhizophora mangle L. Beata; *Howard 12414*. — A coastal shrub most abundant on the north coast.

COMBRETACEAE

Conocarpus erecta L. Beata; *Ostenfeld 316, Howard 12340*. — A coastal plant on mud and marl flats. Trees to fifteen feet tall.

Conocarpus erecta L. var. **sericea** Griseb. Beata; *Howard 12413*. — Locally abundant at the northern end of Beata. Foliage heavily silky pubescent. Plants to ten feet tall.

Laguncularia racemosa (L.) Gaertn. Beata; *Howard 12431*. — Relatively abundant at the edges of the salt pans on the east coast of Beata.

MYRTACEAE

Cryptorhiza haitiensis Urb. Beata; *Ekman* (*August Frett 7063*). *Howard 12345, 12446*. — This species was described by Urban, based on material collected between Cueva Alta and Trujin and Juan Lopez and Trujin on the Barahona peninsula. Reference is also given in the original publication to a collection made by August Frett on Beata Island. I have been unable to learn anything further about the latter man or the nature or amount of collections he made on Beata. *Cryptorhiza haitiensis* is a common three-foot shrub growing on limestone on Beata Island. All plants were sterile in August. However, the distinctive foliage and aroma of the plant, coupled with the common names of cañelito and cañelillo, allowed determination of the collections. This plant was well known to all the sailors who were my companions. A strong aromatic tea made from the leaves

was recommended for upset stomachs or intestinal disorders; however, the same leaves were brewed into a mild and sweetened tea which we all drank with considerable pleasure nearly every evening aboard the boat.

Eugenia aeruginea DC. Beata; *Ostenfeld 300.*

Eugenia buxifolia (Sw.) Willd. Beata; *Ostenfeld 300, 302, Howard 12347.* — A common shrub of twelve feet on the limestone plateau.

Eugenia aff. **fragrans** (Sw.) Willd. Beata; *Howard 12354, 12343.* — An eight- to twelve-foot shrub collected on the marshy ground around the salt pans and on the limestone plateau. Records of this species from Hispaniola are all for higher altitude locations than these from Beata Island.

Eugenia linearis L. C. Rich. Beata; *Ostenfeld 340, Howard 12381.* — A fifteen-foot shrub found on the limestone plateau. This species was very common in restricted locations, with many seedlings and saplings in the vicinity. Urban regarded the Ostenfeld collection as a narrow-leaved form of the species, but the variation of leaf width with the age of the plant was striking in one colony in the field.

Eugenia rhombea (Berg.) Krug & Urb. Beata; *Howard 12350.* — A common shrub, generally about eight feet tall. It occurred on the limestone, but was most abundant in sheltered areas along the western coast of the island.

THEOPHRASTACEAE

Jacquinia barbasco (Loefl.) Mez. Beata; *Howard 12409.* — Bushes of five feet with abundant red fruits were found along the north coast of the island.

Jacquinia linearis Jacq. Beata; *Howard 12441.* — A small bush two to three feet tall was found in the windswept area along the north coast of the island. This species has not previously been reported from the southern coast of Hispaniola.

APOCYNACEAE

Cameraria angustifolia L. Beata; *Howard 12403.* — This species was illustrated by Burman in *Plantae Americanae* in describing Plumier's plants and was described by Linnaeus (Sp. Pl. 210. 1753). Moscoso reports this species was collected by *Robert Schomburgk* (no. *145*), but no location was given for the collection. In 1926 Urban described a new species, *Cameraria linearifolia* (Ark. Bot. **20A** (5): 39. 1926), based on an Ekman collection, which Woodson later reduced to synonymy under *C. angustifolia* (N. Am. Fl. **29**: 121. 1938). Ekman's material came from Morne Cadets in Haiti. To the best of my knowledge neither Plumier nor Schomburgk was on Beata Island, and so this species may well be expected in the Barahona province.

probably in the Enriquillo valley and possibly in the limestone areas south of Trujin.

The material I collected on Beata Island in August came from a twenty-foot tree. Plants were relatively common in local areas along the cliff on the west coast. The trees were in flower and fruit. A description of the mature fruits has been lacking in the literature. The samara-type fruits were paired, each samara being 22–24 mm. long. The lateral wings, located slightly above the median line of the fruit, are strongly veined. The apex of the samara is emarginate when mature and deeply emarginate-cleft in development. The wings are lobed about one third from the base. The lobes are rounded above and cuneate at the base of the fruit. At the broadest part of the wings the fruit is 11 mm. wide.

Echites umbellata Jacq. Beata; *Howard 12506.* — A woody vine becoming shrubby. The attractive white flowers make this a conspicuous plant on the sandy beaches.

Lochnera rosea (L.) Rchb. Beata; Howard by observation. — An introduced ornamental planted near the buildings in the northwest corner of Beata.

Mesechites repens (Jacq.) Miers. Beata; *Ostenfeld 334, Howard 12510.* — A very common vine just back of the sandy beaches and in certain areas of the limestone.

Plumeria obtusa L. (*P. beatensis* Urb., *P. ostenfeldii* Urb.). Beata; *Ostenfeld 249, 341, Howard 12424, 12491.* — A common shrub of the limestone area most abundant in the windswept northern end. The number of specimens representing the two collections I made show sufficient variation to support Woodson's reduction of the Urban species (N. Am. Fl. **29**: 117. 1938).

CONVOLVULACEAE

Calonyction tuba (Schlecht.) Colla. Beata; *Ostenfeld 323, Howard 12394.*

Evolvulus alsinoides L. var. **grisebachianus** Meisn. Beata; *Howard 12395.* — Herbaceous plants of sandy beaches, especially on the west coast.

Ipomoea acuminata (Vahl.) R. & S. Beata; *Ostenfeld 333.*

Ipomoea eriosperma (Desr.) Urb. Beata; *Ostenfeld 343.*

Ipomoea pes-caprae (L.) Sweet. Beata; Howard by observation.

Ipomoea tiliacea (Willd.) Choisy. Alta Vela; *Howard 12463.*

Jacquemontia jamaicensis (Jacq.) Hall. Beata; *Howard 12437.* — An abundant vine in thorn shrub on limestone.

BORAGINACEAE

Cordia buchii Urb. Beata; *Howard 12511, 12495.* — A very attractive shrub of twelve feet with red-orange flowers and yellowish fruits

turning orange. Common on the limestone and occurring on the faces of the cliffs.

Bourreria maritima O. E. Schulz. Beata; *Howard 12378.* — An eight-foot shrub of limestone areas, previously reported only from Cabo Falso and considered a dry-land coastal endemic of Barahona province.

VERBENACEAE

Avicennia nitida Jacq. Beata; *Howard 12412.* — A coastal plant of twelve feet. Most abundant on the north coast.

Citharexylum fruticosum L. Beata; *Howard 12377.* — A shrub of ten feet in height in the thorn shrub.

Clerodendron spinosum (L.) Spreng. Beata; *Howard 12415.* — A low shrub of two feet which seems to occur only in the larger pits in the limestone plateau.

Duranta repens L. Alta Vela; *Howard 12484.* — A common shrub to eight feet in height occurring only on the leeward side of the island.

Lantana reticulata Pers. Beata; *Howard 12391.* — A shrub of three feet occurring on sandy areas.

Stachytarpheta jamaicensis (L.) Vahl. Beata; *Howard 12411.* Alta Vela; *Howard 12473.* A weedy herb of sandy areas near the buildings and the salt pans.

BIGNONIACEAE

Tabebuia ostenfeldii Urb. Beata; *Ostenfeld 342, Howard 12509, 12497.* — A shrub five to six feet tall growing on limestone and especially on the cliff faces. The small grayish foliage coupled with the pale lavender flowers makes this an attractive plant and a rather distinct species. The species is based on the Ostenfeld collection but has also been collected by Ekman on Gonave Island and recorded by him from Anses-à-Pitre on the Barahona peninsula.

RUBIACEAE

Antirrhoea lucida (Sw.) Hook. Beata; *Howard 12382.* — An attractive fifteen-foot tree on limestone.

Antirrhoea elliptica Urb. & Ekman. Beata; *Howard 12418.* A low two-foot shrub occurring abundantly in the low windswept formation at the north end of Beata Island. This species was described from material collected by Ekman on Cabritos Island in Lake Enriquillo. It has not been reported from the Barahona peninsula, and this represents the second record of its occurrence.

Chiococca alba (L.) Hitchc. Beata; *Howard 12367.* Alta Vela; *Howard 12479.* — A common, often stout vine in open thorn shrub throughout the island of Beata. On Alta Vela the species forms a rampant shrub on the lee hillsides.

Ernodea littoralis Sw. Beata; *Howard 12429.*

Exostemma caribaeum (Jacq.) R. & S. Beata; *Howard 12386.* — A fifteen-foot tree in the thorn shrub on the limestone plateau.

Erithalis vaccinifolia (Griseb.) Wr. Beata; *Howard 12416.* — A prostrate woody shrub occurring in large sink areas in the windswept formation at the northern end of Beata Island. The white flowers and shining black fruits make this a particularly attractive plant. This species has previously been known from Cuba. While Standley (N. Am. Fl. 32: 280. 1934) cites Santo Domingo as the known distribution of the species it is not listed by Moscoso, and I have seen no material from this island.

Guettarda stenophylla Urb. Beata; *Howard 12512.* — A five-foot shrub growing on the cliff faces on the western side of Beata Island. Only one previous collection from El Charco del Gato on the Barahona peninsula is known to me.

Guettarda xanthocarpa Britton. Beata; *Howard 12368.* — A fifteen-foot tree in the thorn shrub on the limestone plateau. This species has been collected in several locations on southern Barahona peninsula. Standley refers this species to the synonymy of *G. cueroensis* Britton N. Am. Fl. 32: 236. 1934), but a field knowledge of both species prompts me to retain *G. xanthocarpa*, as it occurs in Hispaniola, as distinct.

Isidorea leonardii Urban. Beata; *Howard 12357.* — A five-foot shrub on the limestone plateau.

Morinda royoc L. Beata; *Howard 12439.* Alta Vela; *Howard 12454.*

Psychotria nutans Sw. Beata; *Howard 12355.* — A six-foot shrub in thorn shrub on limestone plateau.

Randia parvifolia Lam. Beata; *Howard 12370.* — This eight-foot shrub occurred in the thorn shrub on the limestone plateau.

Strumpfia maritima Jacq. Beata; *Howard 12421.* — A common low shrub in the windswept area at the northern end of Beata Island.

COMPOSITAE

Borrichia arborescens (L.) DC. Beata; *Ostenfeld 337, Howard 12422.* — A low shrub occurring at the dried edges of the mangrove swamps.

Eupatorium corymbosum Aubl. Alta Vela; *Howard 12478.* — A very abundant shrub on all areas of Alta Vela.

Tetranthus cupulatus Urb. Beata; *Howard 12443, 12452.* — Previously reported from Paradise on the Barahona peninsula. A white-flowered herb in holes in coastal limestone benches.

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EXPLANATION OF PLATES

PLATE I

FIG. 1. Beata Island as seen from the west, midway of its length.

FIG. 2. Sandy beach on the west coast of Beata Island looking to the south. *Ipomoea pes-caprae* is the vine at the edge of the sand. *Coccoloba uvifera* and *Suriana maritima* form the first shrub zone. The major cliff profile is at the right.

PLATE II

FIG. 3. Grass-covered wind-blown sand zone encroaches on the limestone at the north end of Beata Island.

FIG. 4. A shrub zone on the deeply fissured limestone at the north end of Beata Island. Most of the shrubs in the foreground are rooted in crevices. Palms in the background are *Haitiella ekmanii*.

PLATE III

FIG. 5. Thorn shrub vegetation on the plateau area of Beata Island. The large tree at the left is *Bursera simaruba*.

FIG. 6. A group of tree cacti, *Opuntia moniliformis*, on the plateau limestone of Beata Island.

PLATE IV

FIG. 7. Alta Vela Island as seen from the north in the pass from Beata Island.

FIG. 8. The typical precipitous shore line of Alta Vela Island. A man in the upper right corner is in an area of turks head cactus, *Cactus lemari*.

PLATE V

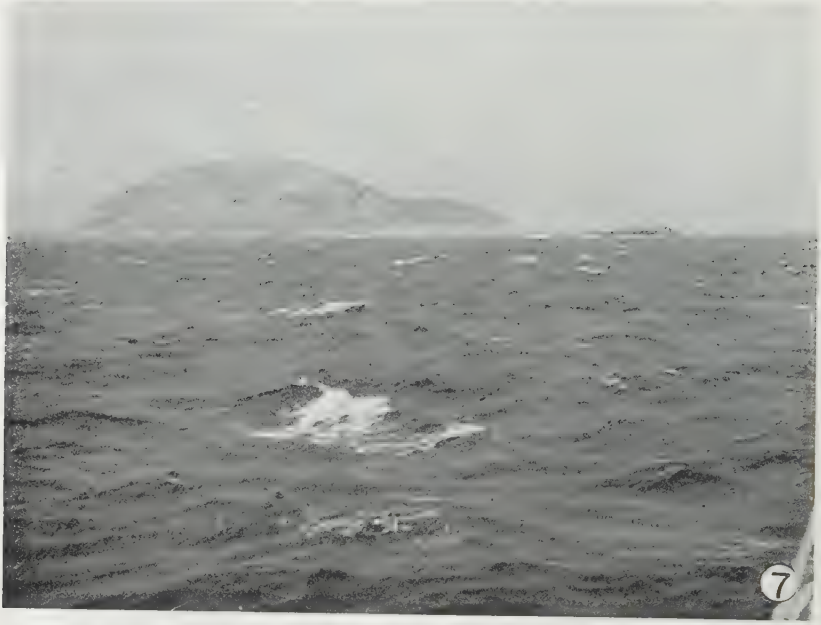
FIG. 9. The light-house tower at the crest of Alta Vela. The wind-swept shrub occurs on limestone boulders.

FIG. 10. The southeast corner of Alta Vela showing the scars of the early guano-mining operations still visible. Shrubs in the lee of the hill at the right are *Capparis cynophallophora*. The grassy area near the shore line hides large stands of *Opuntia antillana*. The destroyer escort vessel "27 de Febrero" stands by off shore.

HOWARD, VEGETATION OF BEATA AND ALTA VELA

Howard, Vegetation of Beata and Alta Vela

HOWARD, VEGETATION OF BEATA AND ALTA VELA



Howard, Vegetation of Beata and Alta Vela

Howard, Vegetation of Beata and Alta Vela

ON THE PRIMARY VASCULAR SYSTEM AND THE NODAL ANATOMY OF EPHEDRA

MARGERY P. F. MARSDEN AND TAYLOR A. STEEVES *

With three plates

THE AFFINITIES of the Gnetales have long been a subject of debate among students of Pteropsid relationships, particularly in regard to possible associations with the Coniferales and with the Dicotyledons. In this type of study the primary vascular anatomy usually figures prominently. *Ephedra* is often referred to as having a two-trace system of foliar vasculature. Marsden and Bailey (1955) recently suggested that the two-trace unilacunar node is a basic pattern in Pteropsida, and they described *Ephedra* as having a eustelic primary vascular system with two traces supplying each leaf from a single gap at the node, and attaching to separate portions of the eustele.

Nägeli (1858) first observed this vascular pattern in *Ephedra*. In subsequent literature, however, there is by no means general agreement about the primary vascular pattern in this genus. Thompson (1912) for example has stressed the importance of a girdle of nodal wood to the extent that he does not consider the internodal bundles to be directly related to the leaf traces, maintaining that the traces arise from this girdle and pass directly out into the leaves at the same node. He has also figured *Ephedra distachya* and *E. altissima* as distinctly bilacunar. Moreover, Strasburger (1872, p. 77) and Graham (1908–9), who are in general agreement with the eustelic pattern described by Nägeli, have reported in certain species an additional bundle between each pair of leaf traces in the internode below their insertion. Graham (1908–9) has further stated that this "complementary" or "accessory" bundle provides additional traces for the axillary bud. His descriptions of bundle fusions in the stem, moreover, do not agree with Nägeli's observations. Thompson (1912) has also reported complementary bundles, but regards them, like the other internodal bundles, as having their origin in the nodal girdle. Monoyer (1937) has,

* The authors are deeply grateful to Professor I. W. Bailey who originally suggested this problem. His guidance, advice, and interest throughout the course of this investigation have been an immense inspiration.

We wish to thank Dr. Adriance S. Foster who generously supplied material of several species of *Ephedra* from the University of California Botanical Gardens. Dr. Thomas W. Whitaker very kindly sent material several times during the course of this study, from the collection at the United States Department of Agriculture Station, La Jolla, California. The authors are indebted to Mr. Robert L. Dressler for assistance in the determination of specimens, and to Messrs. W. Barclay Ray and Ronald Shreve for aid in the collection of material. We are grateful to Mr. Peter M. Ray who gave generously of his time to assist with the preparation of the text and photographs.

in addition, reported that *E. fragilis* has three traces in each leaf, and these extend for one internode below their insertion, before anastomosing with certain sympodial bundles, the nature of which is not clear.

These conflicting views seemed to warrant a re-investigation of the primary vascular anatomy of this genus. This paper reports the result of a study of nine species of *Ephedra* with a view to determining the course of the primary leaf traces, their vertical extent in the stem, and their mode of departure from the stele, particularly in relation to the nodal girdle.

Ephedra is characterized by a marked xerophilous habit, with the leaves reduced to scales, and the young stems photosynthetic (PLATE I: 1). The epidermis is heavily cutinized, the stomata are sunken and the cortex has a mesophyll-like appearance (Plate I: 2, 3, 4). In some species the leaves are opposite and decussate, and in others, they are borne alternating in whorls of three. In both cases the leaves are fused basally to form a sheath at the node. Lateral branches are regularly produced in the axils of the leaves. The stem tends to be ridged and grooved in a manner somewhat suggestive of *Equisetum* and the distinctiveness of the nodes gives the stem a jointed aspect which further emphasizes this superficial similarity. During elongation the base of the internode remains meristematic longer than the upper regions and forms an intercalary meristem. This, according to Graham (1908–9), ultimately differentiates completely leaving in many cases an abscission layer several cells in thickness just above the node (PLATE I: 2) by means of which smaller branches are often shed at the end of the growing season.

MATERIALS AND METHODS

This study has been based on an examination of 6 two-leaved species and 3 three-leaved species of *Ephedra*. TABLE I indicates the origin of the specimens studied. Wherever possible a sample of the material used has been deposited in the herbarium of the Arnold Arboretum. Additional material of uncertain identity has also been examined.

In each case several series of sections of young growing tips were prepared. Serial sections of older material were also examined for comparison. In this way it was possible to follow the course of the leaf traces in the early stages of differentiation, and to evaluate the complications resulting from late differentiation and secondary activity. The distribution of bundles in successive transverse sections of each series was plotted and subsequently the longitudinal course of traces was reconstructed from these diagrams.

Fresh material collected at various times throughout the growing season, from 1948 to 1955, was fixed in formal acetic alcohol, medium chromo-acetic alcohol or Craf III (Johansen, 1940). A modified Zirkle's n-butyl alcohol method for dehydrating refractory plant material was employed and the material embedded by the paraffin-tissuemat method described by Pratt and Wetmore (1951). Transverse and longitudinal serial sections were cut at 8 to 10 micra on a Spencer rotary microtome. Haupts' and

TABLE I

Species	Source	No. of Vascular Bundles in Internode **
Two-leaved species		
Ephedra alata * Dcne.	U.S.D.A. Plant Introduction Station, La Jolla, California	*10*
Ephedra equisetina Bge.	Arnold Arboretum, Jamaica Plain, Mass.; University of Manchester Greenhouse, Manchester, England	*6*, 7, 8
Ephedra gerardiana * Wall.	Arnold Arboretum, Jamaica Plain, Mass.; U.S.D.A. Plant Introduction Station, La Jolla, California	*8*, 9, 10
Ephedra intermedia * Schrenk. and C. A. Mey.	University of Manchester Greenhouse, Manchester, England	*8*, 9, 10
Ephedra major procera * Aschers. and Graebn.	Arnold Arboretum, Jamaica Plain, Mass.	*8*
Ephedra viridis * Cov.	Pipes Canyon, San Gorgonio Mts., San Bernardino County, California	*8*, 10
Three-leaved species		
Ephedra altissima * Desf.	U.S.D.A. Plant Introduction Station, La Jolla, California	*15*, 17
Ephedra californica * Wats.	Morongo Canyon, Riverside County, California	*15*
Ephedra chilensis * Miers.	University of California Botanic Gardens, Berkeley, California (Grown from seed collected by J. West #5098)	*15*, 16

* Indicates that a specimen of the material used has been deposited in the herbarium of the Arnold Arboretum, Harvard University Herbarium, Cambridge, Mass.

** The italicized figures in the third column indicate the number of vascular bundles which occurs most frequently in the internodes of the material examined.

Mayer's adhesives (Johansen, 1940) were used to mount the sections. The slides were usually coated with a 0.5% solution of parlodion in ether, preparatory to staining.

The staining combinations employed were:

(1) Heidenhain's haematoxylin (0.5% aqueous) pre-mordanted in iron alum (4% aqueous), with 1% safranin in 50% alcohol as a counterstain (Jeffrey, 1917).

(2) 1% safranin in 70% alcohol, with picro-aniline blue (1 gm. water soluble aniline blue to 100 ml. of 80% ethyl alcohol with 1 ml. of saturated aqueous picric acid) as a counterstain.

(3) 1% safranin in 70% alcohol, counterstained with a saturated solution of cotton blue in lactophenol.

The parlodion coating was removed with acetone and the sections were cleared in xylol and mounted in damar in xylol in the usual way.

TWO-LEAVED SPECIES OF EPHEDRA

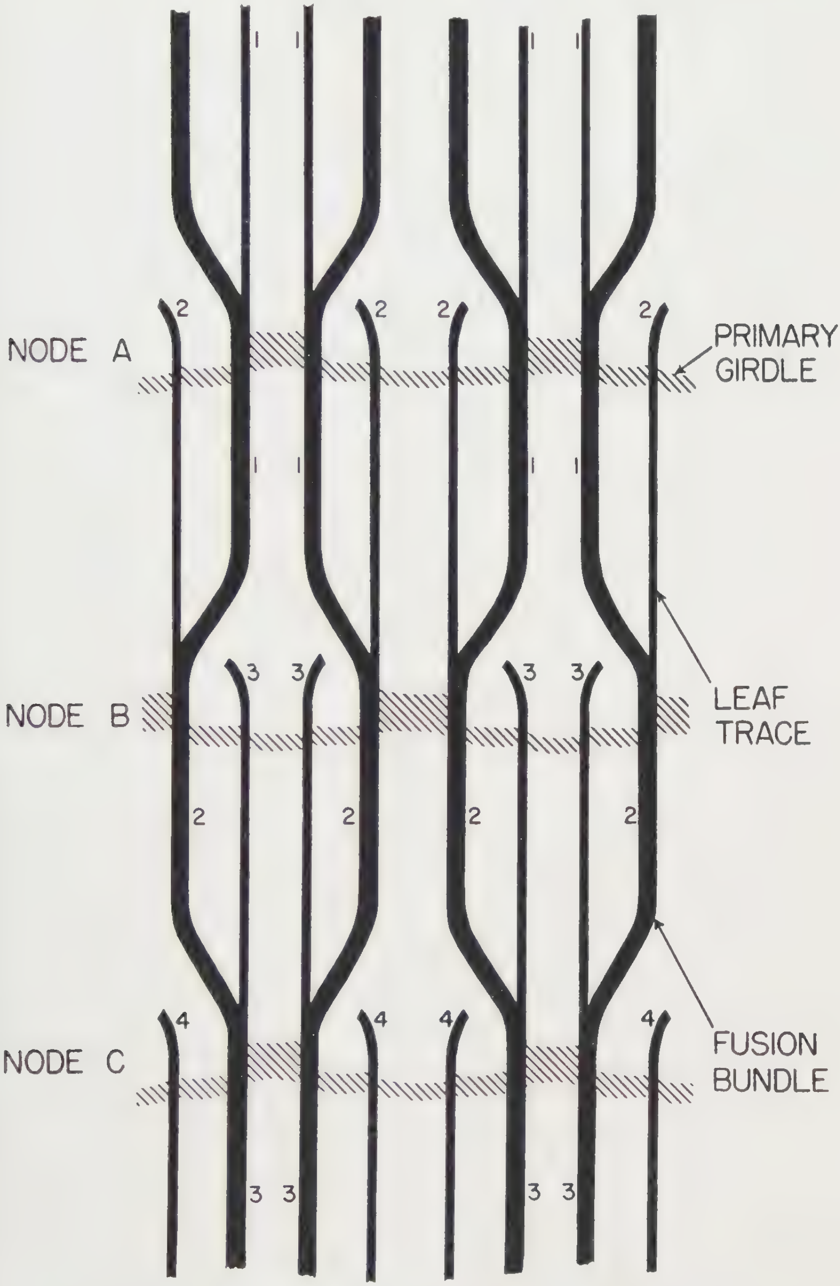
In the range of material examined in this study, a striking uniformity in primary vascular organization was found. It is therefore possible to describe the general pattern for two-leaved species without treating each one individually. Certain deviations from the general plan have been noted; but these represent minor discrepancies, often confined to a portion of a single shoot only.

Each leaf is supplied by two traces which remain separate, as far as could be determined, throughout the leaf. In the internode above a given node (e.g. FIG. 2:6), there are eight bundles in transverse section, two pairs (bundles B) directly opposite the leaves of the node below, and two pairs (bundles C) alternating with these. Just above the node the two bundles of each pair (B) facing the leaves at this node diverge towards the adjacent laterally placed bundles (C) (FIG. 2:7,8); and each fuses with one of these (FIG. 2:9) (FIG. 1 — bundle 2 above node B). As a result of this, there are, directly above the node, four fusion bundles in the transverse section (FIG. 2:9). These become associated in pairs at the node, laterally to the incoming leaf traces, and, through the intercalation of vascular elements, two "lateral crescents" of vascular tissue (FIG. 2:9,10) are formed. The two pairs of traces from the leaves of this node (e.g. traces C, FIG. 2:3; traces D, FIG. 2:10) now attach to the ends of these crescents. At the level of junction, there is no primary xylem between the two traces of each incoming pair (FIG. 2:3,10); but at a slightly lower level the "nodal girdle" is completed by the appearance of vascular tissue in these regions (FIG. 2:5,11). However, at this level the two fusion bundles in each lateral crescent (e.g. B in FIG. 2:5) have begun to separate into distinct strands.

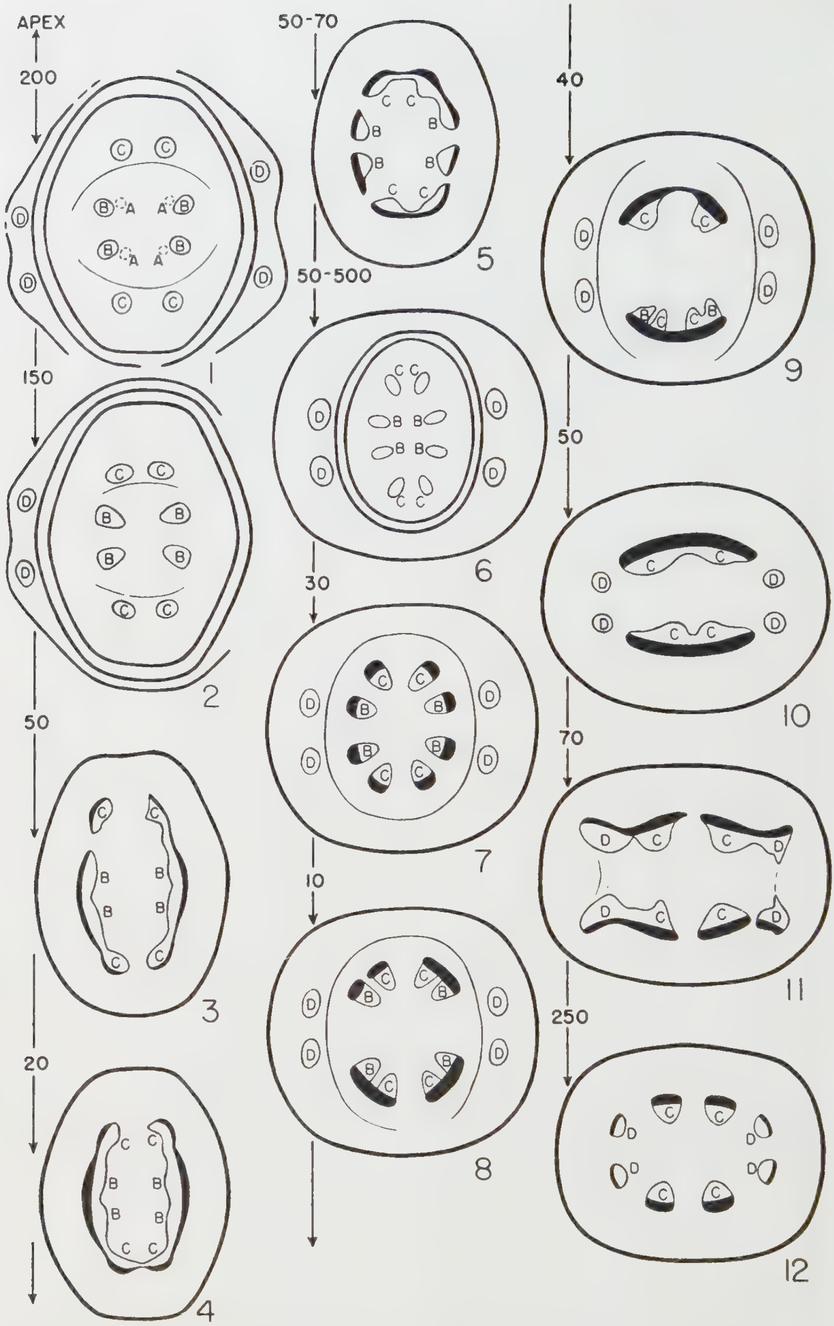
Below the node 8 distinct bundles are again present (FIG. 2:12). The two pairs of traces (D) from the leaves of the node above have continued through the nodal girdle, and alternate with two pairs of somewhat larger fusion bundles (C), which have also extended through the nodal girdle. The arrangement is much the same as that above the node, except that the pattern has been turned through 90° (cf. FIG. 2:6 and 12) (PLATE II, cf. 1 and 5).

Ephedra is characterized by the development at an early stage of conspicuous axillary buds. The several bundles in these buds unite laterally to form two traces in their course through the cortex of the main axis. These traces join to the lateral crescents of the nodal girdle on each side just above the level of leaf trace attachment. They appear to join the traces in these crescents rather than the incoming leaf traces, as has been suggested by Graham (1908–9).

From the description given above, it is apparent (FIG. 1) that the two



TEXT-FIGURE 1. A diagram of the longitudinal course of the primary vascular strands through three nodes in the stem of a two-leaved species of *Ephedra*. The diagram is constructed to show the strands spread out in one plane. The extent of primary xylem in the nodal girdle is shown by striping.



TEXT-FIGURE 2. A series of transverse sections through a young stem of *Ephedra viridis*. Procambium is shown within the dotted lines. The area within the solid lines represents the extent of the primary xylem. Secondary tissue is shown in solid black. No attempt has been made to show the primary phloem. Consecutive sets of two pairs of vascular bundles entering the stem as leaf traces are designated by letters in sequence. The distance in micra between sections is shown on the left.

traces (e.g. traces No. 2) from any given leaf, after entering the stele, extend vertically through the internode below. Just above the next node (B) they (traces No. 2) are joined by the bundles of the leaves two nodes above (traces No. 1). Since the combined bundles subsequently follow the same vertical course as the original traces (No. 2), and since the bundles which join them (No. 1) diverge from their course in so doing, it seems reasonable to consider these combined bundles as further extensions of the leaf traces. This terminology would appear to be in keeping with that of Esau (1943) for *Linum*. The traces (No. 2) extend through the node (B) and the succeeding internode; and, just above the next node (C) diverge, each joining with a trace (No. 3) from a leaf one node above (B) (that is, one node below that at which they themselves pass out into the leaves). Each leaf trace thus has a vertical extent in the stem of nearly two internodes; and the two traces from any one leaf join the traces of two separate leaves from the node below. It is apparent from the reconstruction of FIGURE 1 that there are in the stems of two-leaved species of *Ephedra* four essentially independent vascular systems, and that the two traces of any given leaf are derived from two of these.

This interpretation of the primary vascular system of *Ephedra* is essentially in agreement with that given by Nägeli (1858), Geyler (1867–68, p. 196) and Strasburger (1872). It differs from that of Graham (1908–9) in that he believed that the leaf traces, after a vertical course of two internodes in the stem, fuse with the traces of the leaves which are attached at the second node below that of their own departure from the stele. There is, however, an even sharper divergence between the interpretation given here and that of Thompson (1912), who held that the leaf traces arise directly from the girdle at each node. He did, however, indicate that in some cases a local thickening of the girdle suggested a weak connection between a leaf trace and a bundle in the internode below. The difference of opinion clearly rests upon an interpretation of the nodal girdle.

In an examination of mature regions of the stem it is difficult to determine the continuity of bundles through the nodal girdle. However, if one examines immature regions of the stem, the phenomenon is easier to observe. In all species examined there was no doubt that the leaf traces, at the node of their insertion, formed only a transitory association with the girdle, never lost their identity in it, and continued as distinct bundles in the internode below. At the node below, after being joined by the traces of leaves from one node above their own departure, the fusion bundles on each side appear to merge to form the lateral crescents (PLATE II:3). In some cases it is difficult to follow them through the lateral crescent. However, in other cases, particularly in immature material, it is clear that they do extend through the crescent, and this view is enhanced by the fact that the corresponding large bundles in the internode below coincide in position with the fusion bundles just above the node (PLATE II: cf. 2, 3 and 5).

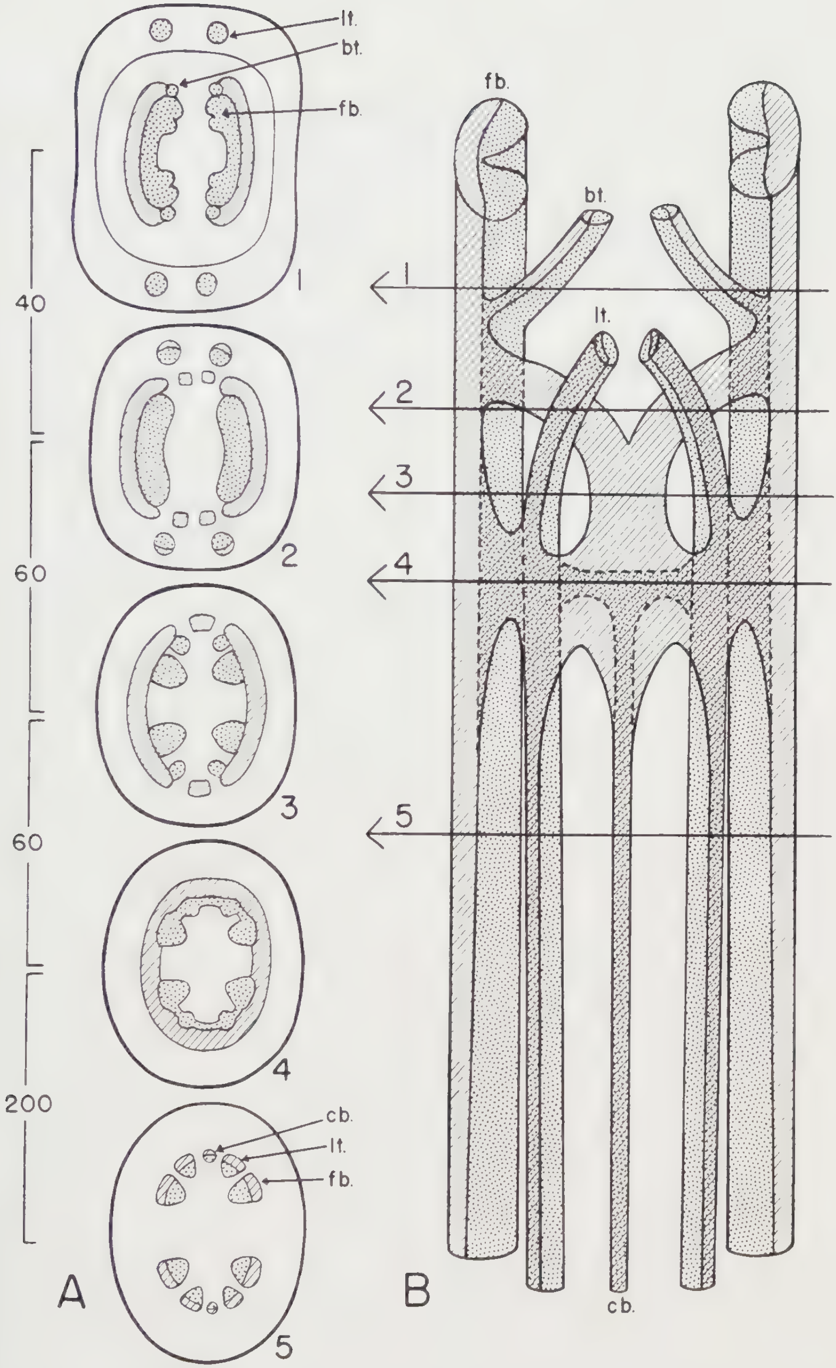
An examination of longitudinal sections of the node shows the nature of the girdle (PLATE I:2). It is composed for the most part of relatively

isodiametric tracheids somewhat resembling transfusion tissue. The vascular bundles appear, however, to be continuous through this tissue. The elements in the bundles are broader and shorter than those in the internodal portions; but they are distinctly longer than those of the girdle wood between bundles (PLATE I:2). This broadening of the elements of the bundle is particularly pronounced in any leaf trace at the node below its insertion, that is when it is a fusion bundle. This explains the difficulty in tracing the fusion bundles through the lateral crescents.

The structure of the nodal region is complicated by an additional phenomenon, the early initiation of cambial activity. Cambial activity has been observed as close to the apex as the third node in some cases, although frequently it is later in its appearance. It is of interest that secondary activity is initiated separately at each node, and subsequently spreads through the internodes above and below. Cambial activity soon superimposes a heavy nodal girdle of secondary tissue upon the primary structure and adds to the difficulty of tracing bundles through the node.

Secondary tissue alters the appearance of the departure of the leaf traces and has led to some confusion. The primary tissue of the nodal girdle is apparent between the two traces of a given leaf only below their attachment to the lateral crescents. Above their departure there is a single gap, and the node is distinctly unilacunar (PLATE II:3). Cambial activity, in addition to thickening the girdle between the two recently attached traces, spreads upward between them at an early stage forming a mass of secondary wood at and above the point of departure (FIG. 3). In mature nodes of *E. viridis* this "bundle" of secondary wood was observed to split above the level of departure of the leaf traces, one half attaching to the secondary portion of the lateral crescent on each side (FIG. 3), just below the departure of the two branch traces. Thus the two leaf traces appear to have separate gaps as figured by Thompson (1912), but these gaps are in the secondary body and the only vascular tissue between the departing traces is secondary. This condition in *Ephedra* has been referred to by Sinnott (1914).

Although the basic primary vascular system of two-leaved species of *Ephedra* provides for eight bundles in the internode, it is apparent (Table I) that considerable variation occurs. Variation may even be noted in different portions of the same branch. This variation was observed by earlier workers, notably Strasburger (1872), Graham (1908–9), and Liu (1929). A reduction from the basic number of eight, particularly common in *E. equisetina*, occurs by the formation of what Graham (1908–9) has called "concrescent" bundles. After their association in the lateral crescent at the node below their insertion, the two leaf traces of a given pair fail to separate into distinct bundles, and are represented during the second internode of their vertical course by a single bundle only. The occurrence of this phenomenon on one or both sides of the axis gives rise to the 6- or 7-bundle condition (see PLATE I:3). At the node below, where the two traces normally diverge, the concrescent bundle usually divides into two



TEXT-FIGURE 3. Diagrams of transverse sections and longitudinal course of primary (stippled) and secondary (striped) vascular tissues of a mature node of *Ephedra viridis*. Only the vascular strands on the near side of the stem (which is the side where a leaf is inserted) are shown in the longitudinal diagram. The distance in micra between the sections in A is shown on the left. fb. fusion bundle; bt. = bud trace; lt. = leaf trace; cb. = complementary (accessory) bundle. *Note* the positions of the primary and secondary girdles.

parts, one part fusing with a trace on each side. However, less frequently, the entire bundle diverges to one side and fuses with one trace.

The occurrence of 9 or 10 bundles requires a different explanation. This is provided by the "complementary" or "accessory" bundles noted by Strasburger (1872) and by later workers. Located between the two traces of a leaf, this complementary bundle extends from the nodal girdle where the leaf traces enter the stem to the lateral crescent between these two traces at the node below. It does not appear to anastomose with the leaf traces at any level. Accompanying one or both pairs of leaf traces, such bundles produce the 9- and 10-bundle condition (PLATE I:4).

THREE-LEAVED SPECIES OF EPHEDRA

The three-leaved species examined in the course of this study exhibit a degree of uniformity comparable to that of the two-leaved species. The general pattern is similar to that of the two-leaved species, but involves a larger number of bundles. The increased number of bundles results from the presence of an additional leaf at each node, and from the regular occurrence, in the three species examined, of complementary bundles. Graham (1908–9), however, reported that the complementary bundle does not always occur in three-leaved species of *Ephedra*.

Typically, in the internode above a given node, fifteen bundles are seen in transverse section (PLATE III:1). Three groups of three bundles, each group consisting of the pair of traces from a leaf of the first node above accompanied by a complementary bundle, alternate with three pairs of somewhat larger bundles, which are the leaf traces of the leaves of the second node above. In the upper regions of the stem the complementary bundles are frequently not yet differentiated, so that only twelve bundles are present in the internode (FIG. 5:3). Just above the node, the paired bundles which are opposite the leaves of this node diverge and each fuses with the trace adjacent to it, so that there are now three pairs of fused bundles, each with a complementary bundle between the two members (PLATE III:2). Each pair is then joined by vascular elements of the nodal girdle, forming three crescents lateral to the incoming leaf traces (e.g. FIG. 5:6). In immature nodes, the crescents are often not present (PLATE III:2,3), and the fusion bundles remain distinct through the node. The complementary bundles terminate in the crescents without joining either leaf trace, as in the two-leaved species having extra bundles. The incoming traces take up their position in the stele and form a transitory connection with the lateral crescents (FIG. 5:6,7) (PLATE III:3). In many cases the bundles in the crescents separate before the connections of the incoming traces are formed. At a slightly lower level, below the insertion of the traces, a band of nodal xylem is apparent between the two traces (FIG. 5:7,8) (PLATE III:4). There is thus a complete nodal girdle, but it is not seen as such at any one level since the connections between the fusion bundles usually do not occur at the same level as the connections between the incoming traces.

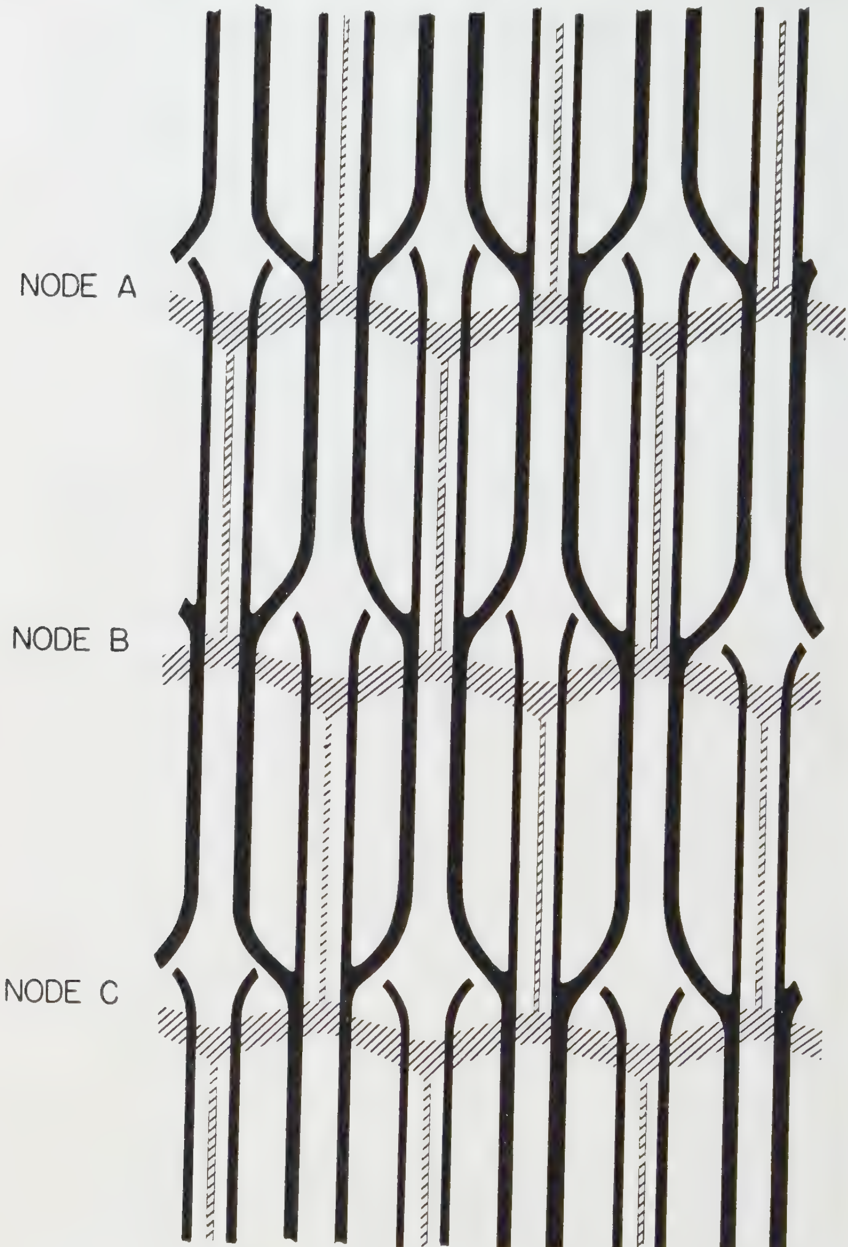
Below the node, the bundles are again distinct, with six pairs of larger bundles clearly representing the fusion bundles from above the node, and three groups of three smaller bundles which are the leaf traces of the last node, each pair accompanied by a complementary bundle (FIG. 5:9). The complementary bundle originates in the nodal ring between the traces. Occasionally extra complementary bundles are formed (FIG. 5:9) so that sixteen or more bundles may be present in the internode.

It is apparent from this description that, as in the two-leaved species, each leaf trace has a vertical extent of nearly two internodes in the stem (FIG. 4). It is joined at the second node by the trace of a leaf one node above its own insertion, and at the third node itself joins the trace of a leaf one node below its own insertion. There are, in the stems of the three-leaved species of *Ephedra*, six independent vascular systems; each leaf is supplied by traces derived from two of them. The three-leaved species are, like the two-leaved, unilacunar, there being no primary vascular tissue between the two traces at or above the level of insertion. (PLATE III:3). The early appearance of cambial activity complicates the nodal structure, forming a heavy ring of secondary tissue. Secondary activity spreads upwards between the departing leaf traces, forming between them a band of secondary wood in mature nodes. The departing traces then leave two gaps in the secondary body.

The regular occurrence of a complementary bundle makes possible a more detailed consideration of its structure and significance than was possible in the two-leaved forms. The complementary bundle has been observed repeatedly extending from nodal girdle to nodal girdle without in any way joining the leaf traces. However, in several cases in *E. altissima*, individual leaves having three traces were observed. In all cases the median trace was continuous below with the complementary bundle. The behavior of the lower portion of this bundle under these conditions was in no way different from the normal condition. The significance of this three-trace leaf vasculature is not clear. Monoyer (1938) has reported three traces in the leaves of *E. fragilis*. Graham (1908–9) states that the complementary bundle extends upwards through the node, divides, and provides accessory vasculature for the lateral bud. It seems probable that this author has misinterpreted the secondary tissue between the departing traces (cf. FIG. 3) as an upward extension of the complementary bundle.

DISCUSSION

The primary vascular system of *Ephedra* seems best interpreted as a eustele in which the internodal bundles are continuous with the leaf traces. The rather anomalous occurrence of a girdle of nodal wood, through which the leaf traces must be considered to pass, poses certain objections to this interpretation. Thompson (1912) has in fact concluded that the leaf traces cannot be followed through this girdle. At an early stage of differentiation, however, the continuity of strands through the girdle seems quite clear, and the additional nodal vascular elements have the appearance of



TEXT-FIGURE 4. A diagram of the longitudinal course of the primary vascular strands through three nodes in the stem of a three-leaved species of *Ephedra*. The diagram is constructed to show the strands spread out in one plane. The extent of primary xylem in the nodal girdle is shown by striping. *Note* the complementary or "accessory" bundles (also shown striped).

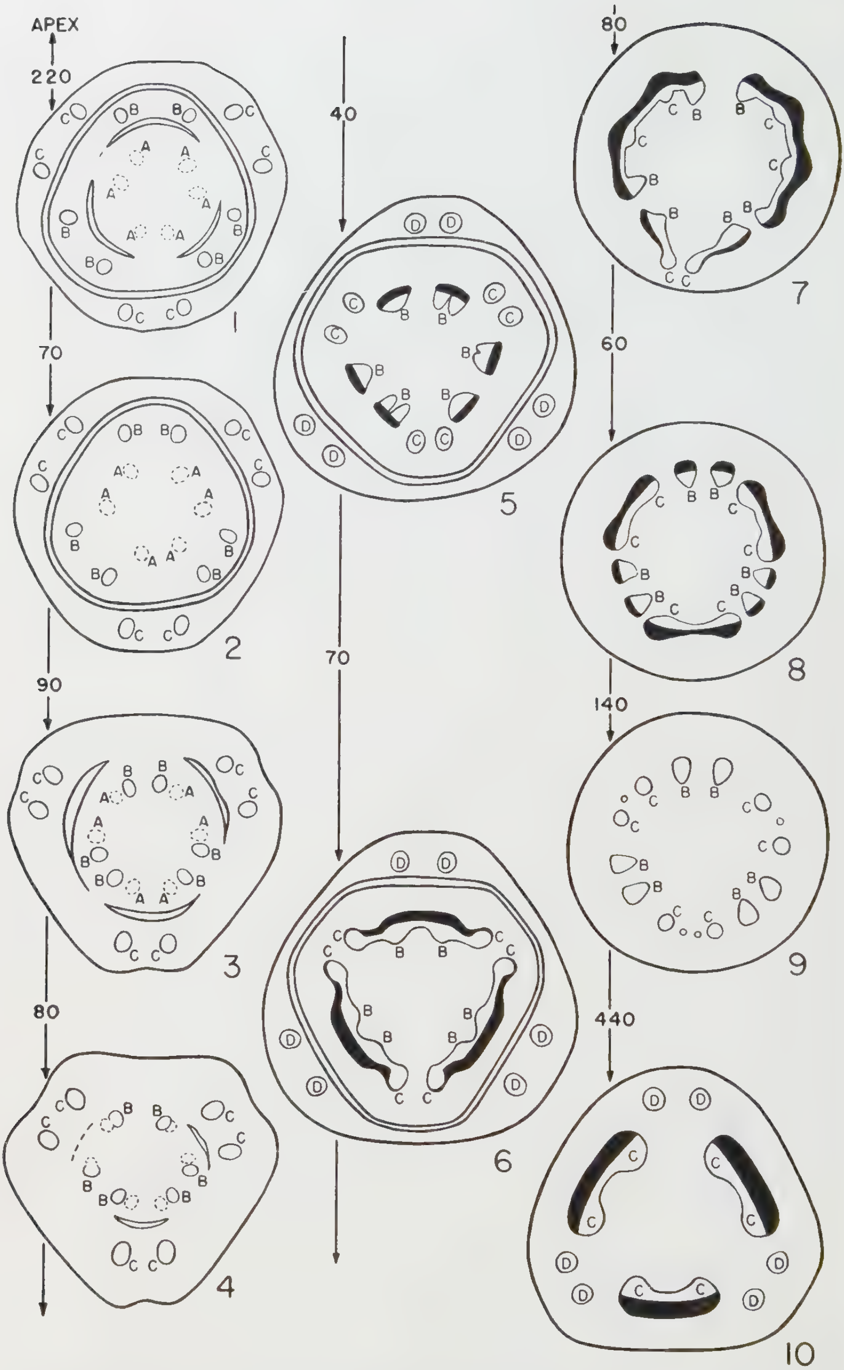
being intercalated between the leaf traces. This appearance is striking, in spite of the relatively early differentiation of the girdle, because of the peculiar form of its elements. These are nearly isodiametric in shape and bear a strong resemblance to those of transfusion tissue. The bundles can be traced through this girdle in longitudinal section by the relatively elongate nature of their tracheary cells. In the nodal region, however, even the elements of the bundles are somewhat shorter and wider than they are in the internodal region. Possibly this general shortening of elements at the node is related to the non-elongation of this region, as compared to the extensive elongation of the internodes. The nodal girdle would appear to be a specialized feature superimposed upon the basic eustelic pattern, possibly in relation to some physiological necessity.

The superficial resemblance of *Ephedra* to *Equisetum* in external characteristics is paralleled, as many workers have noted, by a similarity of internal organization, particularly in the occurrence in both of a nodal ring of relatively short vascular elements. It is interesting, and perhaps significant to the present study, that the interpretation given here of the relation of the leaf traces to the nodal girdle closely resembles that offered by Golub and Wetmore (1948) for *Equisetum*, on the basis of a developmental study.

Further support for this interpretation of the primary vascular system of *Ephedra* is provided by the close correlation between the leaf number and arrangement at the node and the bundle pattern in the internode below. The difference in internal organization between two- and three-leaved species is clearly related to the difference of leaf number. Moreover, Bertrand (1874, p. 20) reported that in material of *Ephedra triandra* having four leaves at each node, there are eight pairs of bundles in the internode. This is the pattern which would be expected for a stem bearing four leaves at each node, assuming that there are no complementary bundles. Graham (1908–9) has further stated that in stems in which the leaf number changes from two to three, corresponding differences may be noted in the internodal bundle pattern. In the present study no material possessing four leaves at the node has been observed, nor have any stems showing changes in leaf number been encountered.

On the other hand, the occurrence of complementary bundles extending from the girdle at one node to the girdle at the next, would seem to disprove a close correlation between the leaves and the internodal vascular bundles. Except in rare cases in which the complementary bundle extends into the leaf as a third trace, it has no connection with the leaf traces. The complementary bundles would appear to be, like the nodal girdle, an accessory structure superimposed upon the basic eustelic pattern. This view is supported by the relatively late differentiation of complementary bundles in comparison with the leaf traces. They are often not distinguishable in any form in the first several internodes at the tip of a stem, even though the leaf traces are clearly recognizable at this level (see Fig. 5:3).

In the two-leaved species of *Ephedra*, there are four quite separate vas-



TEXT-FIGURE 5. A series of transverse sections through a young stem of *Ephedra chilensis*. Procambium is shown within dotted lines. The area within the solid lines represents the extent of the primary xylem. Secondary tissue is shown in solid black. No attempt has been made to show primary phloem in the strands. Consecutive sets of three pairs of vascular bundles entering the stem as leaf traces are designated by letters in sequence. *Note* the appearance of complementary (accessory) bundles in section 9. The distance in micra between sections is shown on the left.

cular systems. Each of these four systems supplies half the vascularization of one of the pair of leaves at every node. Similarly in the three-leaved species there are six separate vascular systems, each of which contributes half the vascular supply to one of the three leaves at every node. These vascular systems are basically similar to those exhibited by a number of other plants, particularly among the Gymnospermae and Angiospermae. In *Ginkgo biloba*, for example, it has been reported (Gunckel and Wetmore, 1946a and b) that there are two distinct foliar traces supplying each leaf in both long and short shoots. These traces depart through a single gap in the stele. Gunckel and Wetmore (1946b) figure the connection of each foliar trace to an independent bundle of the eustele.

Prof. I. W. Bailey and his students have demonstrated the occurrence of this two-trace unilacunar condition in several ranalian families, for example, in the Austrobaileyaceae (Bailey and Swamy, 1949), Monimiaceae (Money, Bailey and Swamy, 1950) and Chloranthaceae (Swamy and Bailey, 1950; Swamy, 1953). Further, this nodal condition is known to occur widely throughout other dicotyledonous families (Marsden and Bailey, 1955) in both the adult and seedling conditions.

In *Austrobaileya*, the opposite leaves are supplied by two pairs of strands through unilacunar gaps in the stele (Bailey and Swamy, 1949). The two foliar traces are independent from one another below the node and arise from two different strands of the eustele. As in the two-leaved species of *Ephedra*, there are four separate vascular systems. Similarly, in *Trimenia*, four separate vascular systems supply the decussately arranged leaves. Each leaf is vascularized by two distinct traces which depart through a unilacunar gap in the stele as in *Ephedra*, *Austrobaileya* and *Ginkgo*; and the leaf traces arise from independent strands in the eustele. Similar conditions have been recorded for *Ascarina*, *Hedyosmum* and *Chloranthus* (Swamy, 1953).

The leaf traces of *Ephedra* have been figured as having two separate gaps at the node (Thompson, 1912; Sinnott, 1914). It has been shown in the present investigation that this "bilacunar" condition is a superimposed one since it occurs only in the secondary vascular tissue and not in the primary body. A similar condition occurs in *Trimenia*. At the node in *Agathis spinulosa* and *Mesoxylon sutcliffii* (Thompson, R. B., 1913) the two foliar traces are separated by a wedge of secondary wood. Thus in all of these cases the node basically shows a unilacunar, two-trace condition.

The vascular pattern in *Ephedra* poses a number of significant questions which might be answered by a developmental study. An understanding of the unusual nodal structure of this genus can only be obtained from an investigation of the development of the nodal girdle in relation to the leaf traces which extend through it. The possible importance of the non-elongation of the node in this connection has already been mentioned. Similarly, the early and separate initiation of cambial activity at the nodes is a phenomenon which deserves further study, particularly in view of the profound influence of this secondary growth upon the structure of the node. The delayed differentiation of the complementary bundles has been noted

earlier. A thorough study of the differentiation of the complementary bundle, including cases in which it appears to become incorporated into the leaf vasculature as an extra trace, would seem to be required. A developmental study of *Ephedra*, particularly directed along these lines, is now being undertaken.

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EXPLANATION OF PLATES

PLATE I

1. Habit of *Ephedra viridis* growing in the greenhouses of the Harvard Biological Laboratories.

2. Longitudinal section of a node of *Ephedra* sp., showing the nature of the elements of the nodal girdle and the continuity of a bundle through it. The bundle on the left is a leaf trace passing through the lateral crescent at the node below its departure from the stele. The short girdle elements appear to project towards the center because of the obliquity of the section (× 70).

3. Transverse section of an internode of *Ephedra equisetina* showing the six-bundle condition (× 100).

4. Transverse section of the stem of *Ephedra gerardiana* showing ten bundles in the internode (× 70).

PLATE II

SERIES OF TRANSVERSE SECTIONS THROUGH AN IMMATURE NODE OF *Ephedra gerardiana*.

1. Internodal section with eight bundles (× 70).

2. Just above the node showing the divergence of the traces of leaves two nodes above, towards the traces of the leaves one node above (× 70).

3. Section at the node, with two lateral crescents and paired leaf traces attaching at their ends. Note evidences of cambial activity in the crescents (× 70).

4. A slightly lower level showing a nearly complete nodal girdle. The traces in the lateral crescents have begun to separate. Note evidence of cambial activity (× 70).

5. Below the node, with eight distinct bundles again present (× 70).

Plate III

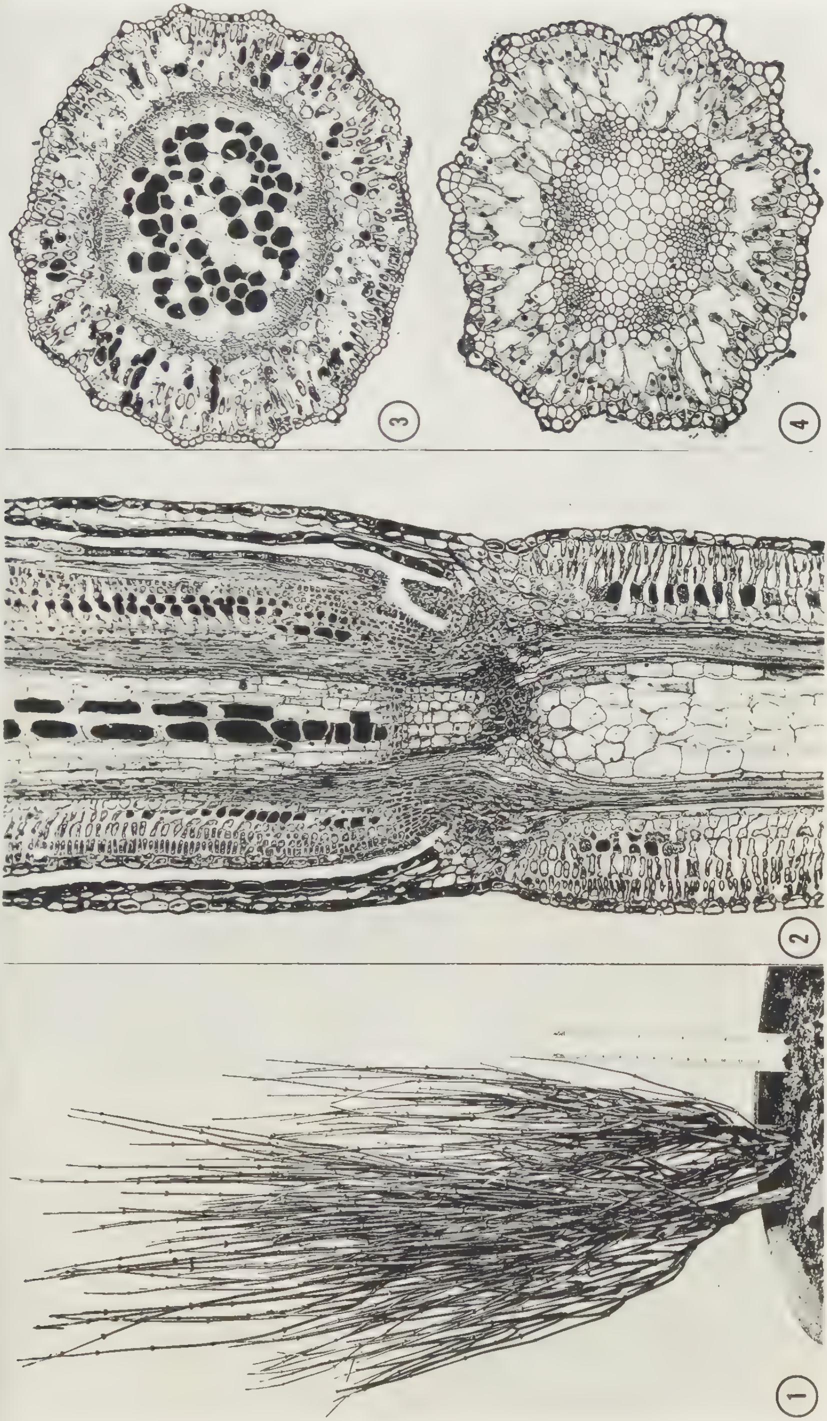
Series of transverse sections through an immature node of *Ephedra altissima.*

1. Internodal section with fifteen bundles (× 60).

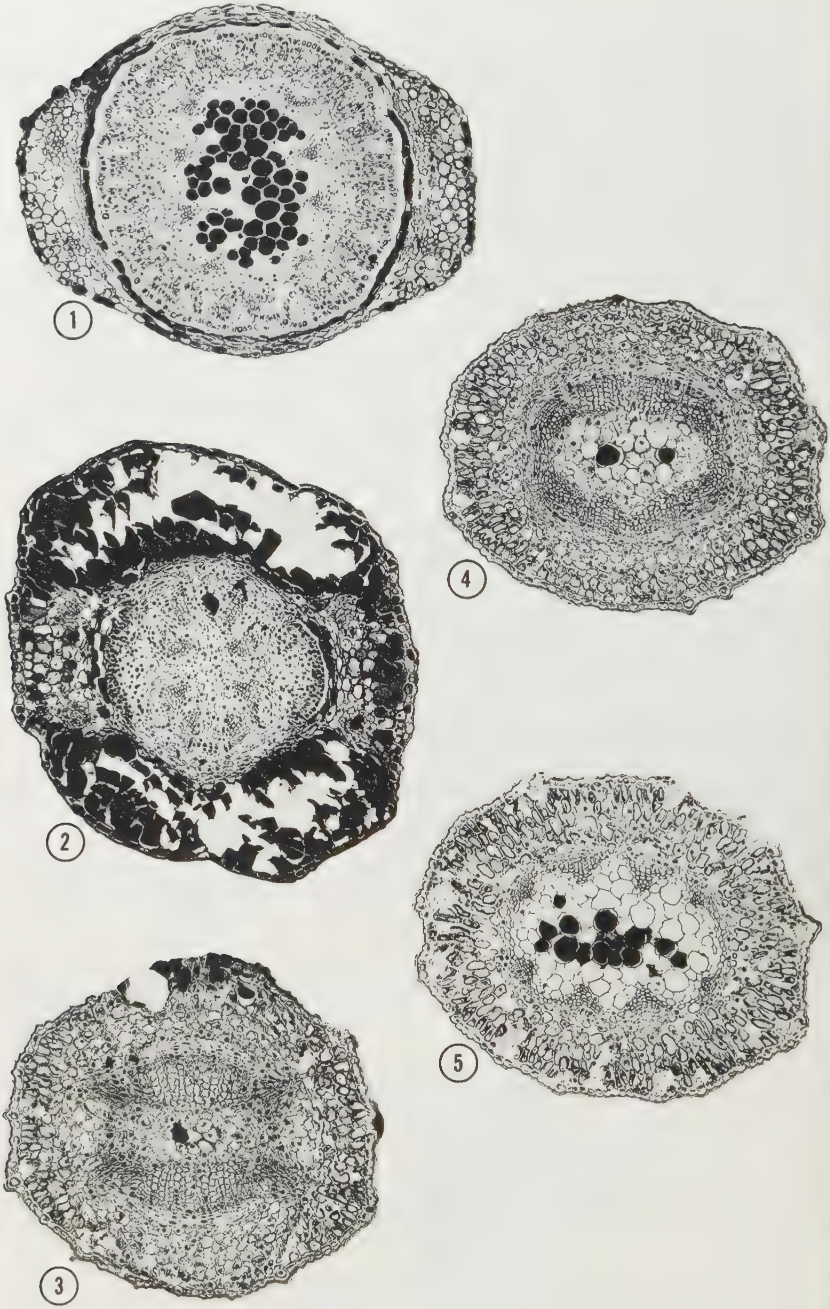
2. Just above the node showing fusion of traces from leaves two nodes above with traces of leaves one node above. Note the complementary bundles (× 60).

3. Nodal section showing the attachment of incoming leaf traces. In this immature region of the stem no lateral crescents have been formed (× 60).

4. A slightly lower level, showing bands of primary xylem between pairs of recently attached leaf traces (× 60).



Marsden and Steeves, Anatomy of Ephedra



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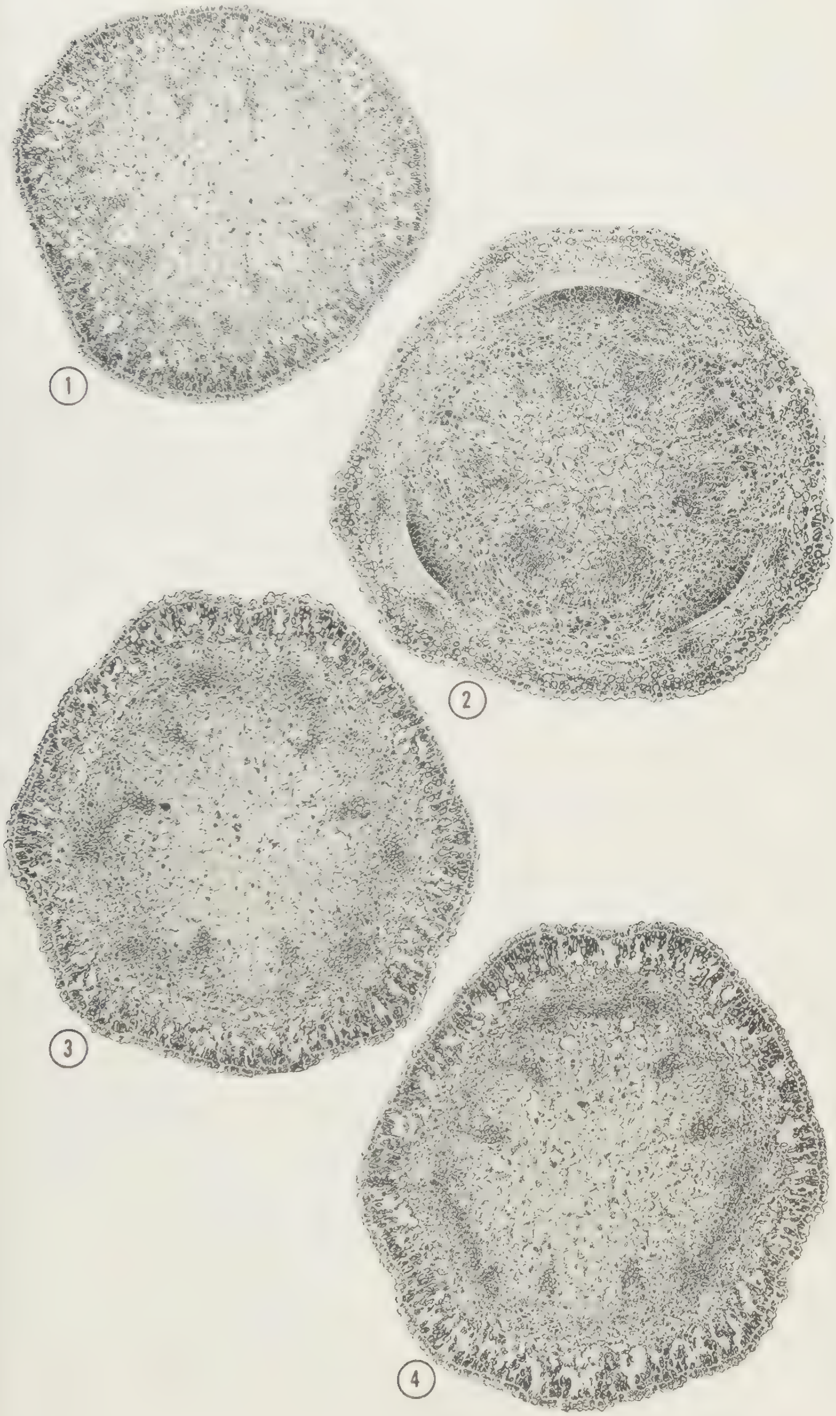

Marsden and Steeves, Anatomy of Ephedra

THE OCCURRENCE OF EUPTELEA IN THE CENOZOIC OF WESTERN NORTH AMERICA

RICHARD A. SCOTT AND ELSO S. BARGHOORN

With three plates

IN THE EOCENE CLARNO FORMATION of north central Oregon logs and numerous fragments of silicified wood occur in association with fruits and seeds. The Clarno formation, one of a series of early Tertiary deposits in the basin of the John Day River, consists of interbedded conglomerates, tuffs, ash layers and lava flows. In very limited outcrops of the formation, in beds of siliciously cemented volcanic tuff, numerous seeds and fruits may be found. These are preserved chiefly in the form of chalcedony filled locule casts, and retain in minute detail the internal morphology and anatomical structure of a wide range of angiosperm seeds and fruits. The seed flora, insofar as it has been studied, has been described and interpreted in an intensive investigation by Scott (1954 and unpublished mss.). The tissues of the silicified logs and more abundant fragments of silicified wood which accompany the seeds and fruits are remarkably well preserved in many cases, and in both external form and internal organization the logs and fragments show evidence of silicification prior to compression failure from the weight of overlying sediments. The bulk of the preserved stems are decorticated, and commonly, in thin section, show evidence of extensive fungus attack on the wood, hence indicating conditions of aerobic decomposition preceding burial and mineralization.

The Clarno woods are being intensively studied with regard to their botanical identification and interpretation, and a number of genera have now been determined. Among the genera thus far recognized is one of unusual interest from the standpoint both of its morphology and its geographic distribution, viz., the genus *Euptelea*. As far as the authors are aware this occurrence comprises the first fossil record of the genus. The existence of *Euptelea* in the early Tertiary of western America constitutes another significant addition to the growing body of paleobotanical evidence relating the early Tertiary forest history of North America to that of southeastern Asia.

Euptelea is one of a group of small or monotypic genera extant today only in warm temperate and subtropical eastern Asia. Other members of this morphologically and geographically isolated group of woody plants include *Cercidiphyllum*, *Trochodendron*, *Tetracentron*, and *Eucommia*. These five genera have been variously associated taxonomically in the past (Swamy and Bailey 1949), but comprehensive anatomical study has revealed numerous and significant divergences in structural organization and floral morphology among them, demonstrating convincingly that each

genus is best regarded as constituting a distinct family (Tippo 1940; Nast and Bailey 1945; Nast and Bailey 1946; Swamy and Bailey 1949).

The genera constituting the Trochodendraceae, Tetracentraceae, Cercidiphyllaceae and Eupteleaceae possess in common certain ranalean characters in one or more of their organs or parts. The vesselless Trochodendraceae and Tetracentraceae are closely related, having many morphological features in common. On the other hand, the Cercidiphyllaceae and Eupteleaceae are quite distinct from each other and, likewise, quite distinct from the vesselless *Trochodendron-Tetracentron* complex. The genera included in these four families logically may be regarded as relics of ancient ranalean origin which have evolved independently at least since Upper Cretaceous time, and probably will ultimately be found to have been distinct in older Upper Cretaceous deposits. That three of these genera, excluding *Euptelea*, have been in existence in post Cretaceous time as essentially "modern" entities is well documented by their occurrence in Tertiary deposits. *Cercidiphyllum* is known from the Middle Eocene of England, the Eocene of Greenland and from numerous horizons in the early and middle Tertiary of continental United States (Brown 1939). Vesselless dicotyledonous woods conforming in structure to either or both *Trochodendron* and *Tetracentron* have been found in the Eocene of Greenland (Mathieson 1932), the Oligocene of Oregon (Hergert and Phinney 1954) and the Miocene of Washington (Beck 1941, 1944). Fruits apparently belonging to *Trochodendron* have been described from the Eocene of England (Reid and Chandler 1933).

Hence, with the notable exception of *Euptelea*, there is well documented evidence that these presently Asiatic genera once grew on the American continent and were quite probably Holarctic or circumboreal in distribution. Recognition of *Euptelea* among this assemblage of phylogenetically and geographically isolated primitive angiosperms adds measurably to our understanding of the diverse character of the woody flora of early Tertiary temperate and high latitudes in North America and accentuates the degree of depauperization which took place during the late Cenozoic. It is quite probable that further additional components of the highly diversified woody angiosperm flora currently surviving in warm temperate Asia will be found among the early Cenozoic fossil floras of temperate America. Indeed, the question may be asked whether many of these genera are not already known as leaf impressions, but are concealed in fossil floras by mis-applied binomials or form generic names, as was clearly demonstrated by Brown (1939) in the case of the American Cenozoic record of *Cercidiphyllum*.

Assignment of the fossil wood under consideration to *Euptelea* is as follows:

Euptelea baileyana Scott & Barghoorn sp. nov.[1] (PLATE I, FIG. 1; PLATE II, FIG. 1; PLATE III, FIGS. 1, 2, 3.)

[1] This species is named in recognition of Professor Irving W. Bailey's contributions in establishing the morphological and taxonomic position of the phylogenetically isolated genus *Euptelea*.

VESSELS: Small, mean tangential diameter 54 μ, somewhat smaller in the late wood; chiefly solitary, occasionally paired, or appearing paired as a result of overlapping of vessel elements; about 85 per sq. mm. in the early wood, somewhat more abundant in the late wood. Thin walled; oval or slightly angular in transverse section. Vessel elements up to 0.85 mm. long. Perforation plates oblique, exclusively scalariform; bars mostly narrow and widely spaced, varying in number from 5 to 35, most commonly about 15, per perforation plate. Intervascular pit pairs predominantly scalariform with narrow apertures, occasionally opposite. Pits to parenchyma scalariform, the apertures wider than those of the intervascular pits. Numerous thin walled tyloses present. Vessel distribution graded porous.

PARENCHYMA: Very scanty diffuse.

RAYS: Heterogeneous (Kribs Type IIA); multiseriate rays up to 18 cells wide and 2 mm. high, with 1 to 6 marginal rows of enlarged isodiametric or upright cells; uniseriate rays 2 to 7 cells high, composed chiefly of upright cells but occasionally including 1 or more almost procumbent cells; up to 8 per mm.

IMPERFORATE TRACHEARY ELEMENTS: Thick walled fiber tracheids, the pits with small but distinct borders.

LOCALITY AND GEOLOGIC OCCURRENCE: SE $\frac{1}{4}$ sec. 27, T. 7 S., R. 9 E., Wheeler Co., Oregon. Middle (?) Eocene, Clarno formation.

COLLECTOR: Richard A. Scott, July 1950.

MATERIAL: A single specimen of silicified, mature, secondary xylem measuring approximately 2 x 2 x 2 cm. The curvature of the growth rings indicates that the stem, of which this specimen represents a small fragment, had a diameter of at least 10 cm.

HOLOTYPE: University of Michigan, Museum of Paleontology, No. 32338, represented by three ground sections. Three ground sections are also deposited in the Paleobotanical Collections, Harvard University, No. 55572.

AFFINITIES AND DISCUSSION

Structural features of the fossil wood indicate, after extensive comparison, that its closest affinities are with the modern genus *Euptelea*. There is close agreement in all details except those noted below. The fossil wood shows a superficial resemblance to the mature secondary xylem of *Platanus*, particularly as seen in the transverse plane of section. However, the broad rays of *Platanus* are homogeneous, whereas the fossil possesses conspicuously heterogeneous multiseriate rays.

The secondary xylem of the two living species of *Euptelea*, *E. polyandra* Sieb. & Zucc. and *E. pleiosperma* Hook f. & Thoms. is characterized by small, predominantly solitary vessels. There is a conspicuous difference in diameter between the vessels of the early wood and those of the late wood (PLATE I, FIG. 2). The size and distribution of the vessels of *E. baileyana* agree with the distributional pattern in the two modern species. Mean tangential diameter of the vessels in the fossil specimen is intermediate between the measurements secured from comparable secondary xylem

of the two modern species, as follows: *E. polyandra* 52 μ, *E. baileyana* 54 μ and *E. pleiosperma* 57 μ. Thin walled tyloses, present in the vessels of both living and fossil species, appear more abundantly in the fossil. These structures tend to tear out in the sectioning and manipulation of unimbedded wood and hence for this reason there is artificially introduced a slight difference in appearance of the wood of the fossil and living forms.

The two major differences between the vessel elements of the modern and fossil species are in the structure of the intervascular pit-pairs and in the number and spacing of the bars in the scalariform perforation plates. In both the extant species the intervascular pitting is transitional between scalariform and opposite-multiseriate, whereas in the fossil form it is chiefly scalariform. As pointed out by Nast and Bailey (1946), scalariform pitting tends to predominate in the *inner* rings of the stem wood of *Euptelea*, while opposite-multiseriate pitting is characteristic of the larger vessels in the *outer* rings of the older stemwood. However, vessels with scalariform pit pairs occasionally occur in the old wood of *E. pleiosperma*. Phylogenetically, opposite intervascular pitting is reasonably to be interpreted as evolutionarily more advanced than is scalariform pitting among woody angiosperms. In this respect the fossil species is less highly specialized than the two living species of *Euptelea*.

In the proposed new species of *Euptelea* the perforation plates of the vessel elements, however, commonly exhibit a more specialized structure than those of the two living forms. Both *E. polyandra* and *E. pleiosperma* are characterized in the mature secondary xylem by numerous fine bars (20–90) in their vessel perforation plates. The vessel perforation plates of *E. baileyana* usually possess from 5 to 35 bars, most frequently about 15. Also, in contrast to the living species the individual bars are more widely spaced (PLATE III, FIG. 2).

Very little wood parenchyma is present in the fossil *Euptelea* and its presence can be detected only because of the unusual degree of structural preservation. In living species of *Euptelea*, the wood parenchyma is distributed apotracheally and varies from fairly abundant to extremely scanty. In the range of material examined in this study it was observed that wood parenchyma is less abundant in *E. pleiosperma* than in *E. polyandra*. Terminal parenchyma, noted by Metcalfe and Chalk (1950), was not observed. The apparent extreme paucity of wood parenchyma in the fossil may be explained by a variety of factors. One of these is the difficulty of distinguishing between the effect produced by the loss of cell wall substance from the secondary walls of the tracheary elements and the true thin secondary wall and correspondingly larger lumen of parenchymatous elements. Owing to the differential rate of degradation of the various lamellae of the secondary wall various histological effects are produced in fossil plant residues which must be interpreted with this factor in view (Barghoorn 1952).

The largest ray observed in our thin sections of *Euptelea baileyana* is 18 cells in width. In *E. pleiosperma*, which has wider rays than *E. polyandra*, the largest ray observed was 13 cells in width. This is not a large

quantitative difference with respect to this structural feature in comparing the living and fossil species. The frequency of broad, multiseriate rays in the fossil wood, however, is considerably greater than that occurring in either of the two extant species of *Euptelea*. The material of *E. pleiosperma* examined, however, does not represent wood of ring development directly comparable with that of the fossil, being from wood formed in more central rings. Inasmuch as there is a marked increase in ray width during ontogeny of the woody cylinder (Barghoorn 1940) it would be desirable to compare ray width from rings of comparable radii. Wood from the outer rings of large stems of *E. pleiosperma* might show the presence of a higher proportion of large multiseriate rays. Unfortunately, material for determining this feature has not been available.

The close agreement in major diagnostic features as well as in numerous microscopic details of anatomical structure provides convincing evidence that the Clarno fossil wood is closely related to *Euptelea*. Determination of the degree of relationship necessitates careful evaluation of the three observable differences between the Eocene and the modern wood. These differences are limited to 1) the nature of the intervascular pitting, 2) the fine structure of the vessel end walls and perforation plates and 3) the proportion and size of the broad multiseriate rays. In each of these categories, the range of structural variation between the living species of *Euptelea* and the proposed fossil species is less than that which may be found, in numerous instances, among species of a single living genus. For example in *Magnolia* and *Carpinus*, among other genera, the vessel perforation plates vary from scalariform to simple. In *Platanus* and in many genera of the Lauraceae, as examples which may be drawn from a wide range of the dicotyledons, both scalariform and simple perforation plates may be observed in a single radial section. Phylogenetically, these structural differences are more quantitative than qualitative, and in our interpretation of the fossil wood we are extending the morphological range of *Euptelea* rather than proposing a new generic category of the Eupteleaceae.

It has been noted that the vessel perforation plates in the fossil *Euptelea* are more highly specialized than those of the living species of the genus, whereas the intervascular pitting gives evidence of a lesser degree of specialization. This apparent contradiction, however, does not seriously prejudice our assignment of the fossil form to *Euptelea*. Rather, it illustrates the observations of Bailey (1944), drawn from comparative study of the woods of the dicotyledons as a whole, viz., that the phylogenetic modifications of structural features of the vessels of the dicotyledons proceeded at more or less independent rates.

On the basis of close agreement in wood structure the Clarno fossil wood is assignable to the genus *Euptelea*. This genus occupies an isolated position, taxonomically, and possesses secondary xylem of characteristic structure. The possibility that a comparable combination of structural features has arisen quite independently in other families of the more primitive angiosperms seems extremely remote, although this possibility has been considered. Our species has been placed in an extant genus since in our

opinion no useful purpose would be served by clouding its affinities through appending "*-oxylon*" to its generic name.

The occurrence of *Euptelea* as a component of the early Tertiary forests of western United States strengthens the growing evidence that our early Cenozoic floras show stronger affinities to the woody flora now occupying southeastern Asia than has been generally recognized. It serves also to demonstrate the extreme complexity in generic composition of the early Tertiary floras and the difficulties inherent in interpreting them in terms of present day distributional patterns and associations of woody plants.

SUMMARY

A specimen of silicified wood from the Eocene Clarno formation of north central Oregon has been identified as an extinct species of the Asiatic genus *Euptelea*. The wood differs from that of the two living species in only three minor structural details: 1) character of the pitting of the vessel walls; 2) fine structure of the scalariform perforation plates of the vessel end walls; 3) occurrence of a larger proportion of broad multiseriate rays. These differences are quantitative and not qualitative in the evolutionary specialization of secondary xylem; the authors regard them as extending the morphological range of the genus rather than the basis for proposing a new genus. Accordingly, the specimen has been designated as an extinct species of *Euptelea, E. baileyana*. This represents the first fossil record of the genus and establishes its former existence in North America.

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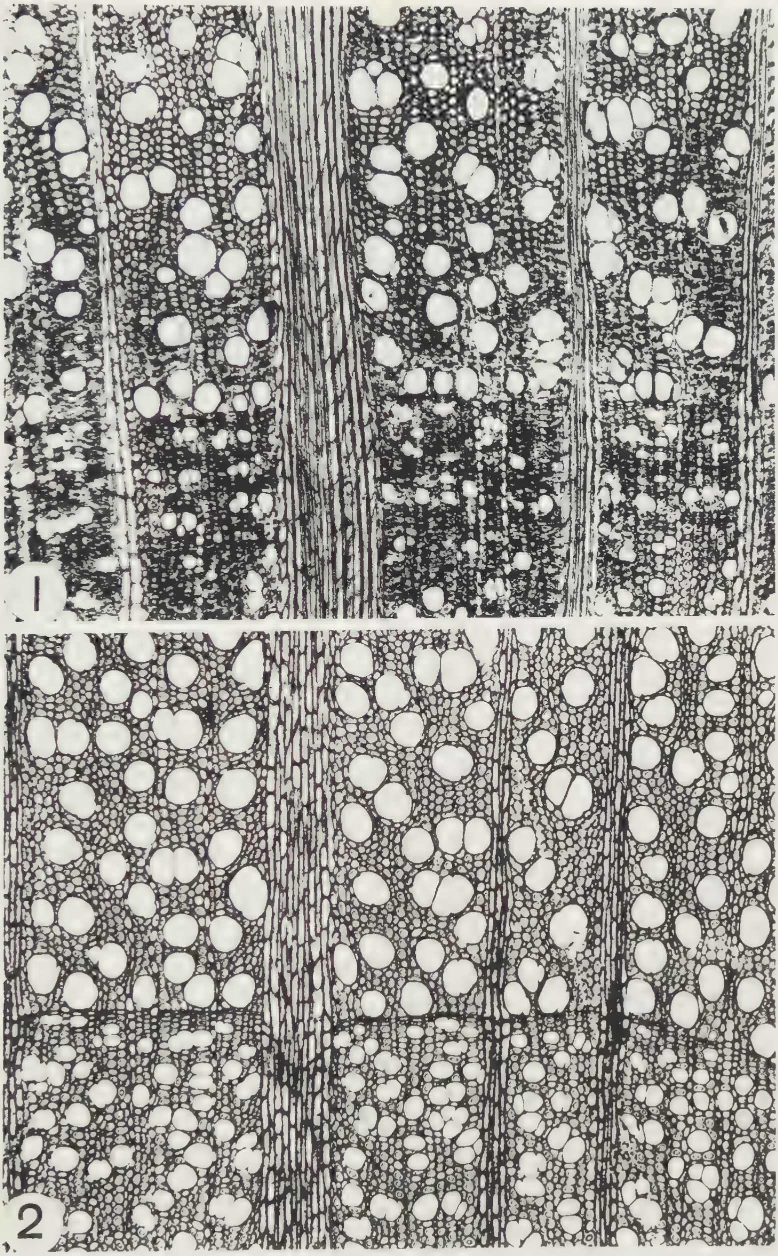
EXPLANATION OF PLATES

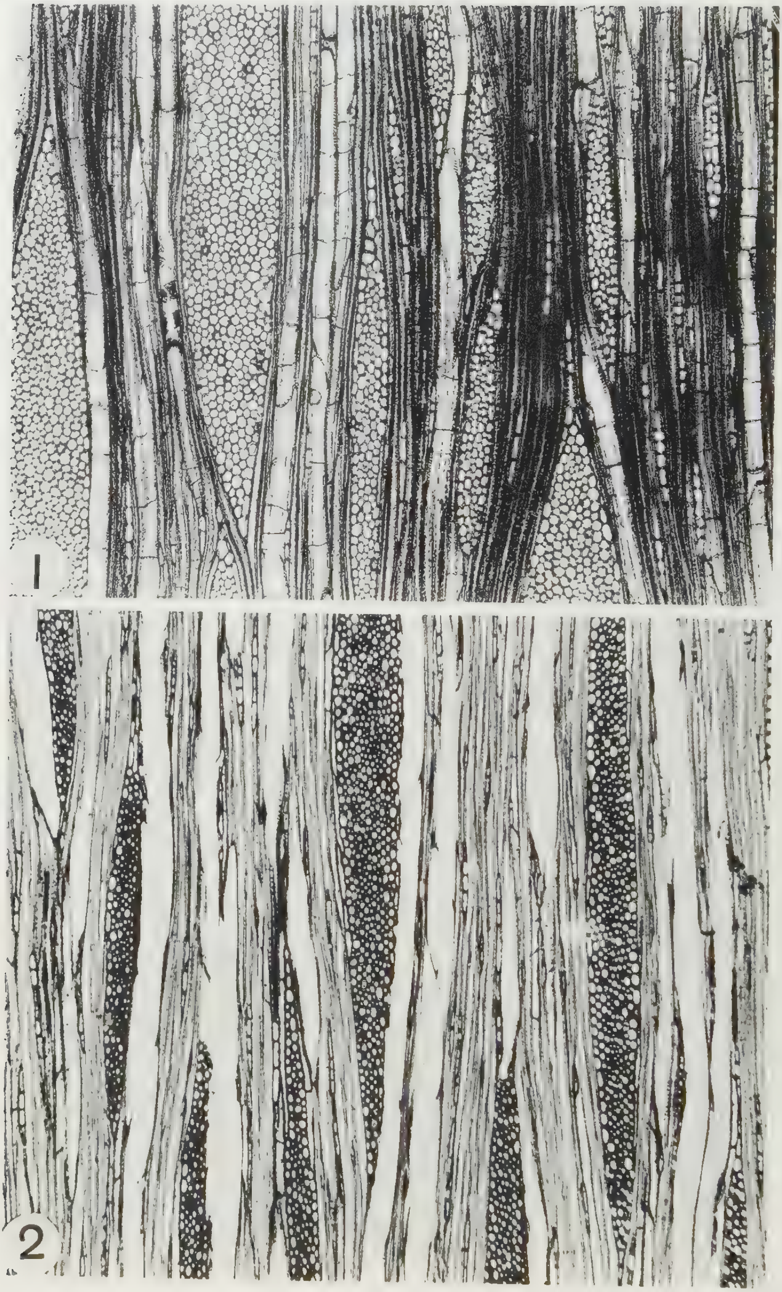
PLATE I. FIG. 1. *Euptelea baileyana* Scott & Barghoorn sp. nov. Transverse section of the wood. × 50. Note the close agreement in major structural features to the modern wood shown below. FIG. 2. *E. pleiosperma* Hook. f. & Thoms. Transverse section of the wood. × 50.

PLATE II. FIG. 1. *Euptelea baileyana*. Tangential section of the wood. × 50. Note the larger proportion of broad multiseriate rays than in the modern wood shown below. FIG. 2. *E. pleiosperma*. Tangential section of the wood. × 50.

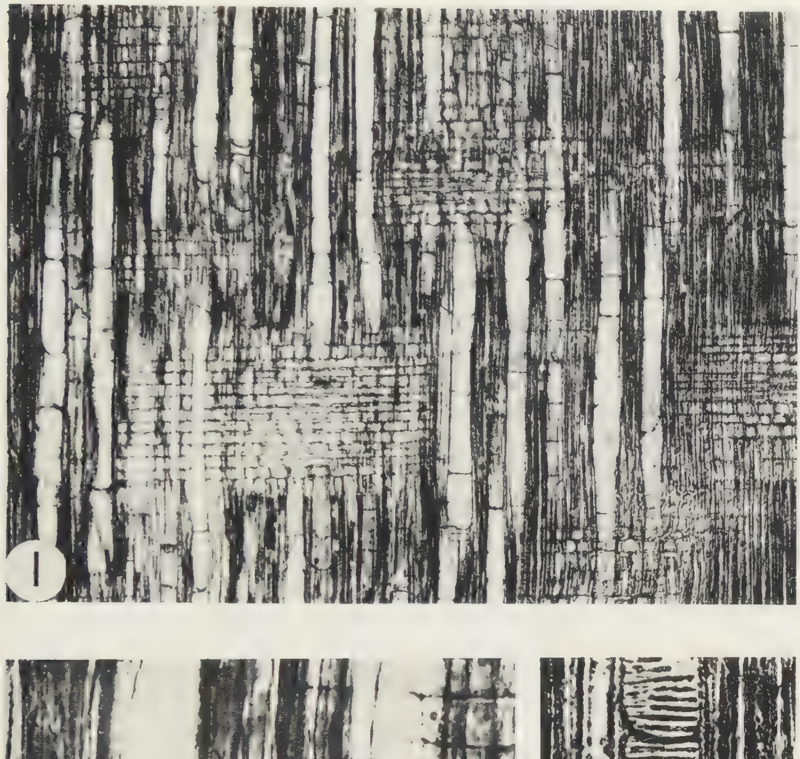
PLATE III. FIG. 1. *Euptelea baileyana*. Radial section of the wood. × 50. Note the numerous thin walled tyloses in the vessels. FIG. 2. *E. baileyana*. Radial section of the wood showing scalariform perforation plates. × 250. FIG. 3. *E. baileyana*. Radial section of the wood showing scalariform intervascular pitting. × 250.



Scott and Barghoorn, Euptelea in the Cenozoic



SCOTT AND BARGHOORN, EUPTELEA IN THE CENOZOIC



Scott and Barghoorn, Euptelea in the Cenozoic

STALK DIAMETER AS A FACTOR IN FRUIT SIZE

Edmund W. Sinnott

Among those factors determining the size to which a fruit will grow, the diameter of its stalk or pedicel should evidently be considered, for through this structure must pass all the material of which the fruit is composed. In the author's cultures of *Cucurbita pepo* there are genetic races differing greatly in fruit size, ranging from types derived from the Connecticut field pumpkin (Line CF) with a fruit diameter of about 300 mm. to small, roundish gourds of about 50 mm. (Line SRC). The purpose of the present study is to determine whether there is any relation in these races between the stalk diameter and the size of their fruits.

In small races like SRC the stalks are about 5 mm. in diameter at maturity and in large ones like CF, about 20 mm. The ratio of fruit diameter to stalk diameter is therefore not very greatly different in the two types. The ratio of fruit *volume* to cross sectional area of stalk, however, is obviously far greater in the larger types than in the small, the former having about 14 times as much fruit volume per unit cross section of stalk as the latter. On the basis of these figures, stalk diameter would therefore seem to have little limiting effect on the attainment of fruit size. It is obvious that the flow of material through the stalk must be very much faster in the large races. It should be remembered, however, that it takes much longer for the fruit of the large race to reach full size than for that of the small, so that more time is available to deliver material to the growing fruit.

In an attempt to gain more information on this problem, two methods of attack were undertaken: a study of the relative growth rate of stalk to fruit in large-fruited and small-fruited races, and a study of growth rate and fruit size in developing fruits of both types where the cross-sectional area of the stalk was experimentally reduced.

GROWTH STUDIES

Daily caliper measurements were made of stalk diameter and of ovary length and width in more than 50 normally growing ovaries belonging to eight races of *C. pepo* ranging in mature fruit size from a volume of about 60 cc. to one of 10,000 cc. Readings were made to tenths of a millimeter on stalk diameter and small ovary dimensions, and to the nearest millimeter on larger ones. They were begun with the smallest ovary primordia that could be measured without injury, usually about five to eight days before the opening of the flower, and were continued until growth ceased. This study was made on field-grown plants at the peak of their development, during the last two weeks of July and the first two of August.

The relation between fruit size and the rate and duration of growth in this material has previously been described by the author (1945). From the beginning of development until about three to ten days after flowering (depending on the race) growth is exponential, and the logarithms of the dimensions, when plotted against time, fall along an essentially straight line. Growth rate then falls off gradually until it ceases altogether. *Rate*

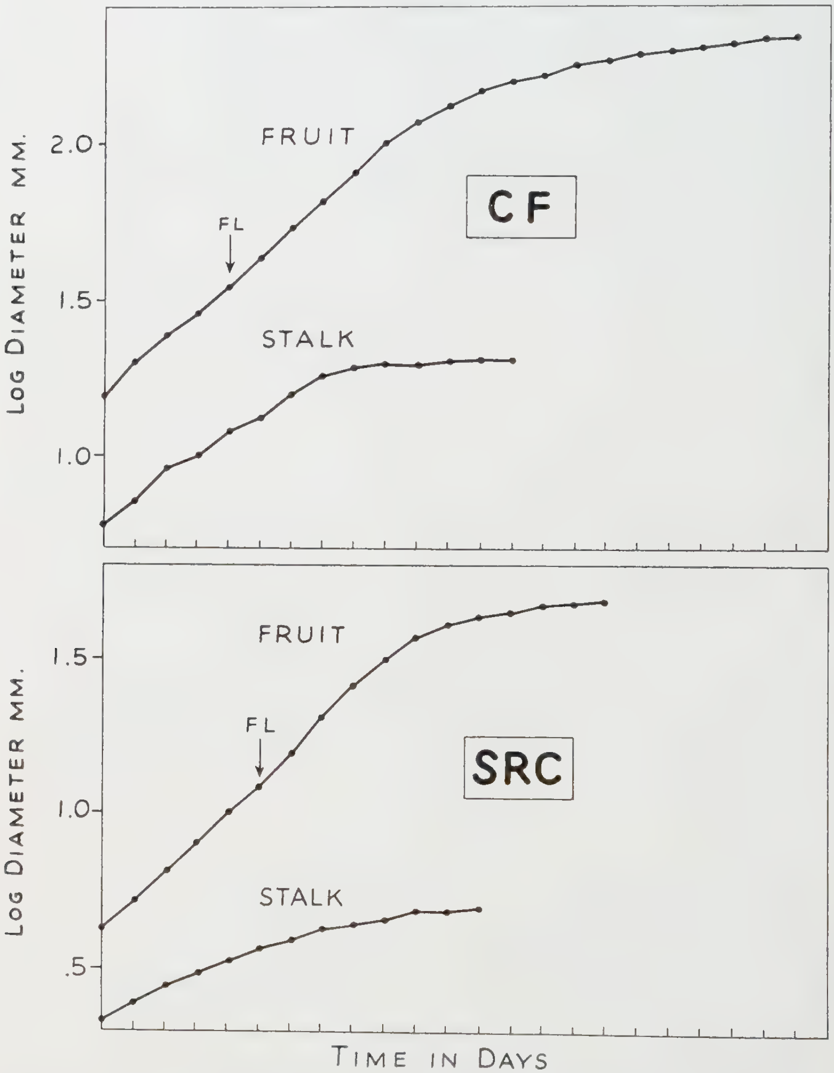


Fig. 1. Growth curves, in days, of the logarithms of diameter of stalk and of fruit in a typical example of a large-fruited line (CF) and of a small-fruited line (SRC). FL marks the day when the flower opened.

of growth during the exponential period is approximately the same in large-fruited and in small-fruited types. The *duration* of both phases of growth, however, is considerably larger in the large-fruited races, and it is to this that the size differences in the mature fruit are due. Typical growth curves for a fruit of a large and of a small race are shown in Fig. 1.

Growth of the stalk in diameter follows the same program as of the ovary, with an early exponential phase succeeded by a gradual diminution until growth ceases. Stalk growth differs in two respects, however, from that of fruit: its exponential growth rate is lower, and its growth ceases much earlier, when the fruit is only one-fourth of its final size or less. These relations are also shown in Fig. 1. As a result, the differential in size between fruit diameter and stalk diameter, which at the beginning is not very great, continually increases.

Between the large and the small races there are differences in these growth relationships. In the large ones the exponential growth rate of the stalk is considerably higher than in the small. In a typical growth curve of the line CF, for example, it is about 16% per day, but in line SRC only 10%. These rates compare with about 22% for daily fruit growth in both. The relative growth of stalk to fruit is thus related to the typical fruit size at maturity. The relative-growth constant of stalk diameter to fruit diameter was determined in the usual manner for six races and is shown in Table 1, together with the typical mature fruit volume of each race.

TABLE 1

Line	Volume of typical mature fruit in cc	Constant of relative growth (k) of stalk diameter to fruit diameter
CF	10,000	.69
P	2,500	.62
AC	680	.65
HG	290	.63
TA	70	.48
SRC	60	.48

At two points on the growth curve there is a relationship between stalk growth and fruit growth in all races which may have morphogenetic significance. One is at the time of flowering. The proportional logarithmic difference between stalk and fruit diameter steadily increases and in both large and small types, when this difference reaches about 5 or 6 logarithmic units, flowering occurs. In other words, the flower opens when the diameter of the young fruit has reached about three to four times that of the stalk. This is quite independent of the absolute size of either. Since the stalk grows faster in large types than in small, it takes longer in them for this absolute differential to be reached, and thus both ovary and stalk are considerably larger at flowering than they are in small types.

The fact is also evident, whatever its significance, that the end of ex-

ponential fruit growth coincides with the end of growth in stalk diameter. Thus all the post-exponential growth of the fruit, during which its volume increases four-fold or more, takes place after the stalk has reached its full size.

EXPERIMENTS

To determine whether the diameter of the stalk actually influences the size to which the fruit will grow, this diameter was experimentally reduced in growing fruits of both large and small types.

In one series this was done by half severing the young stalk by cutting a deep nick out of its side as far as the center of the pith, so that only half of the normal area of the stalk was available for transport of material from plant to fruit. The operation was performed on the day of flowering, or near it. About half the operated flowers stopped growing in a day or two and died, apparently because the cut was too deep, too much sap was lost, or infection set in. In ten fruits, belonging to four lines and differing widely in their normal fruit size, however, active growth continued. The cut surfaces healed well and although considerable scar tissue was formed, vascular connection across the gap was not restored. Daily caliper measurements of ovary width were made on these fruits. In every case where growth continued after the operation it proceeded at the normal rate, and growth curves for these fruits are not different in any notable way from those for intact ones.

Stalk diameter was also reduced by artifically constricting the stalk early in development and thus preventing its growth. Attempts to do this by embedding the stalk in plaster of Paris failed, for the plaster was soon cracked by stalk growth. Constriction was successfully accomplished by winding coarse, soft cotton string around the stalk, firmly but not too tightly, for a length of from several millimeters to a centimeter. In some of the stalks thus bound the string was reinforced by a layer of plaster. Binding was usually done on the day of flowering or shortly thereafter. Young ovaries from six lines were thus constricted.

Many of these failed to grow and died in a few days. Twenty-three continued to develop, however, and width measurements on them were made daily. Growth curves of such fruits, from both large and small races, were essentially normal as to rate and total final size.

After growth had ceased, the stalks were freed and examined. In every case it was found that growth had either been greatly reduced or had not taken place at all after binding. The tissues of the stalk were poorly developed and the vessels were much narrower than in normal stalks. Diameter of the stalk above and below the constriction was a little affected.

DISCUSSION

Experimental evidence shows that a stalk very much smaller in cross section than normal, either because of the removal of part of it or the prevention of its growth, will nevertheless conduct material from plant to

fruit as rapidly and to as large a final amount as a stalk of normal diameter. This confirms the results of some other workers, notably Werner (1931). He found after cutting off all the roots of a maize plant except one small prop root and thus reducing the amount of vascular connection between soil and plant to about one thousandth of its normal cross-sectional area, that the plant grew almost as well as the controls and the amount of transpiration was essentially normal.

Other evidence has shown that the volume of the transpiration stream conducted through a young stem is not related to the amount of xylem which is developed there; in other words that "functional stimulus" of a tissue does not result in its greater development. This is what we should expect from a study of anatomical evidence. In *Osmunda*, for example, the cross sectional area of the xylem of a leaf trace as it leaves the stele is only about 1/8 that which it attains in the rachis above, but this small bundle conducts all the water that the large one does. The large and complex vascular system of a cycad leaf, also, is connected with the vascular axis of the stem by only two small bundles. Evidently in such cases, including the ones here described, the conducting capacity of a structure is considerably higher than it is normally called upon to display, and increased demands upon it do not increase its size.

The close relationship between stalk growth and fruit growth in the material here described, however, and especially the difference in growth patterns between large-fruited and small-fruited types suggests that there may be a correlative growth influence between stalk and fruit which is not related merely to transport of material. The fact that stalk diameter grows more slowly than fruit diameter necessarily results in an increasing tension, so to speak, between the two which might be expected ultimately to affect fruit growth. The further fact that when this growth differential reaches a certain absolute size, flowering takes place in both large and small types, suggests that this differential is in some way related to the attainment of sexual maturity. As the difference grows still greater, another change — the cessation of exponential growth in the fruit — occurs, again suggesting that there is a correlative influence of stalk on fruit. What the physical basis of this correlation may be is unknown. It would be interesting to study the auxin relationships between stalk tissue and ovary tissue in these plants.

We may conclude that there is a definite relation between the relative-growth pattern of the stalk and the fruit in *Cucurbita pepo*, but that the size of the fruit does not depend directly on the cross-sectional area of its stalk.

SUMMARY

Diameters of developing fruits and fruit stalks were measured daily in races of *Cucurbita pepo* differing widely in mature fruit size.

Growth of the stalk is slower and ceases earlier in small-fruited races than in large-fruited ones.

In both, flowering occurs when the ratio of ovary diameter to stalk diameter has reached a specific size.

Artificial reduction of stalk diameter by cutting away part of it or by constricting it had no appreciable effect on fruit growth.

Size of fruit does not depend on cross-sectional area of the stalk but there seems to be a correlation between the growth pattern of the stalk and that of the fruit, which is related to fruit size.

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PHANEROGAM GENERA WITH DISTRIBUTIONS TERMINATING IN FIJI

A. C. SMITH

PROFESSOR I. W. BAILEY (1) has aptly commented on the significance of the Indo-Malaysian region in studies of angiosperm phylogenies. The area delineated by him extends to the southeast as far as New Caledonia and Fiji. In view of the supposed geological boundary of an old Melanesian continent in Fiji (2, 4, 5, 6, 7, 9), as indicated by the occurrence there of continental rock formations, we may well consider whether this region is critical as the terminating point of a significant number of plant groups. An examination of relevant data at the species level would not be very revealing, since in Fiji about 70 percent of the phanerogam species are endemic, while a large proportion of the remaining species have such wide distributions and such efficient means of dispersal that their existing ranges are not enlightening. At the generic level, however, it is possible that the pertinent data will aid in answering the central question, namely whether or not a significant distributional break occurs in or east of Fiji and coincides with the putative boundary between the continental and the oceanic archipelagos. I have already touched on this problem (8), and the present notes are intended as documentation of the conclusions there suggested.

It would perhaps have been advisable to consider the Fiji-Tonga region as a unit, since Tonga is also sometimes supposed to have been marginal on the old continent. The botanical evidence, too, points to a fundamental similarity between the Tongan and Fijian floras, the former being in effect an attenuation of the latter, with very few supplementary taxa at the generic level. The list of genera discussed in this paper would have been somewhat longer if Fiji and Tonga could have been treated together, since several, or perhaps many, genera have ranges terminating in Tonga and therefore omitted from consideration here. A more natural geological boundary would have been the sea between Fiji-Tonga to the west and Samoa to the east, and I believe that the botanical evidence will corroborate this. However, no dependable catalog of the Tongan phanerogam flora is available, whereas an accumulation of notes toward my proposed Flora of Fiji gives me a reasonably complete picture of what phanerogams are now known to occur in Fiji. In the preparation of this paper I have utilized the card-catalog of Polynesian references compiled by Dr. E. D. Merrill, now in the library of the Arnold Arboretum of Harvard University, and have consulted whatever recent revisions are available as well as the herbaria at Harvard and the U. S. National Museum.

There follow, arranged essentially in the Dalla Torre & Harms sequence, discussions of the phanerogam genera that occur indigenously in Fiji but are not found in the island groups farther east. A concluding tabulation and discussion summarize the data.

PODOCARPACEAE

Dacrydium Soland. Pilger (in E. & P. Nat. Pfl. ed. 2. **13**: 239. 1926) mentions *Dacrydium* as a genus of about 20 species; except for one species in southern Chile, its range is Indo-Malaysian. It extends eastward to New Guinea, New Caledonia, New Zealand, and Fiji, where the widespread *D. elatum* (Roxb.) Wall. and the New Caledonian *D. lycopodioides* Brongn. & Gris have been recorded.

Acmopyle Pilger. This small genus is known from two species in New Caledonia and a single endemic species in Fiji, *A. sahniana* Buchholz & Gray (in Jour. Arnold Arb. **28**: 142. 1947).

ARAUCARIACEAE

Agathis Salisb. This genus, with 20 or more species, is distributed from the Malay Peninsula through Malaysia to Australia, New Zealand, New Caledonia, the New Hebrides, and Fiji, where the endemic *A. vitiensis* (Seem.) Drake terminates its range.

TRIURIDACEAE

Andruris Schlechter. In his recent treatment of the Triuridaceae, Giesen (in Pflanzenr. **104** [IV. 18]: 15–29. 1938) recognizes 15 species of *Andruris*, which extends from southeastern Asia and Japan to Micronesia, New Guinea, and Queensland, with the outlying endemic *A. vitiensis* (A. C. Sm.) Giesen in Fiji. The genus does not occur in the islands between Fiji and the New Guinea–Queensland region, but the related *Sciaphila* is in New Caledonia and the New Hebrides.

PALMAE[1]

Calamus L. This vast genus, according to Beccari's revision in Ann. Bot. Gard. Calcutta **11** (1): 1–510. 1908, is distributed from Africa and southeastern Asia through Malaysia to New Guinea and Fiji, where the endemic *C. vitiensis* Warb. terminates its range. More recently the genus has been reported from the New Hebrides.

Neoveitchia Becc. Beccari (in Webbia **5**: 76–79. 1921), realizing that Wendland's genus *Veitchia* (in Seem. Fl. Vit. 270. 1868) included two elements, established the genus *Neoveitchia* for *V. storckii*, leaving *Veitchia* for Wendland's remaining three species and three additional new ones. *Neoveitchia*, in the sense of *N. storckii* (H. Wendl.) Becc., remains a monotypic endemic Fijian genus.

Unfortunately, *Veitchia storckii* is listed as the type species of the conserved generic name *Veitchia* (Internat. Code Bot. Nomencl. 89. 1952). The intention in conserving *Veitchia* H. Wendl. (1868) over *Veitchia* Lindl. (1861) was doubtless to conserve it in the sense of the familiar

[1] The data upon which this discussion of the palms is based were kindly supplied by Dr. Harold E. Moore, Jr., of the Bailey Hortorium.

V. joannis and its immediate relatives. Beccari's prior removal of *V. storckii* from *Veitchia* was apparently overlooked when this species was listed as the genotype. Nevertheless, conservation in this sense is binding until revised, thus leaving *V. joannis* without a valid generic name. In the present treatment we disregard the Code's listing of a type species and return to Beccari's arrangement; a formal proposal to this effect will be made to the next International Congress.

Veitchia H. Wendl. As noted above, we here discuss *Veitchia* in Beccari's sense, as including *V. joannis* H. Wendl. and its immediate relatives, an interpretation that preserves current usage. In this sense, *Veitchia* consists of approximately nine species, of which three are endemic to Fiji. Otherwise there are three or four in the New Hebrides and one in New Caledonia, all endemic, and one known only from cultivated material. *Neoveitchia storckii* (H. Wendl.) Becc. is not a close relative of this group of species.

Vitiphoenix Becc. This endemic genus is represented by eight Fijian species, some being transfers from *Ptychosperma* (Burret in Rep. Sp. Nov. 24: 282–285. 1928) and others more recently described (Burret in Bishop Mus. Occ. Pap. 11 (4): 7–10. 1935; A. C. Sm. in Jour. Arnold Arb. 31: 145. 1950). The genus is very closely allied to *Veitchia* H. Wendl. (in the sense used above) and may eventually have to be combined with it.

Exorrhiza Becc. *Exorrhiza* should probably be included in this treatment, on the basis of Burret's discussion in Notizbl. Bot. Gart. Berlin 12: 591–593. 1935. It is definitely known to include four species, of which three are endemic to Fiji and one to the New Hebrides. A fifth species, *E. vaupelii* Burret, of Samoa, is inadequately known and is suspected by Burret to belong in *Clinostigma*. The relationship of *Exorrhiza* seems to be with *Bentinckiopsis* Becc.

Goniosperma Burret. This endemic genus has two species in Fiji, *G. vitiense* Burret and *G. thurstonii* (Becc.) Burret (in Bishop Mus. Occ. Pap. 11 (4): 10–12. 1935). *Goniosperma* belongs to a group of small, often monotypic, genera extending throughout much of Melanesia, Polynesia, and Micronesia, but apparently absent from the larger islands such as New Guinea. A close relative of *Goniosperma* may be the New Caledonian *Cyphosperma* H. Wendl. rather than *Physokentia* Becc., with which Burret compared it.

Taveunia Burret. This apparently rare endemic genus is known from a single Fijian species, *T. trichospadix* Burret (in Bishop Mus. Occ. Pap. 11 (4): 12–14. 1935). Its relationship, in general, is with the cyphospermate palms.

Goniocladus Burret. This endemic genus is represented by a single Fijian species, *G. petiolatus* Burret (in Notizbl. Bot. Gart. Berlin 15: 87. 1940). *Goniocladus* is another little-known genus that cannot be accurately related at present, beyond placing it among the cyphospermate palms.

ORCHIDACEAE [2]

Cryptostylis R. Br. *Cryptostylis,* a genus of 25 or more species, extends from Ceylon through Malaysia and the Philippines to New Guinea and Australia, with one outlying endemic species in Fiji, *C. vitiensis* Schlechter.

Chrysoglossum Bl. This genus of about 15 species ranges from the Himalayas and southern China through Malaysia and the Philippines to New Caledonia, the New Hebrides, and Fiji, being represented in the last archipelago by three species, of which two (*C. gibbsiae* Rolfe and *C. vesicatum* Reichb. f.) are endemic.

Acanthophippium Bl. This small genus of ten species extends from India and southern China through Malaysia and the Philippines to New Guinea, with a single endemic outlying species in Fiji, *A. vitiense* L. O. Williams (in Am. Orch. Soc. Bull. 10: 169. 1941). The archipelagos between New Guinea and Fiji have not yet disclosed the genus.

Eulophia R. Br. *Eulophia*, a genus of about 200 species, is most abundant in Africa, but it also has a wide distribution in America, Australia, southeastern Asia, and Malaysia, continuing eastward to Micronesia, New Caledonia, the New Hebrides, and Fiji, where its range is terminated by two species, neither endemic.

Grammatophyllum Bl. This small genus, of seven species, occurs through Malaysia and the Philippines, extending eastward to the Solomon Islands and Fiji, where it is represented by the endemic *G. elegans* Reichb. f.

Sarcanthus Lindl. *Sarcanthus*, with 75 or more species, ranges from southeastern Asia through Malaysia and the Philippines to Micronesia and New Guinea, with the outlying endemic *S. nagarensis* Reichb. f. in Fiji. I do not find records of the genus in the groups between New Guinea and Fiji.

Chroniochilus J. J. Sm. Only two widely separated species are known of this little genus, *C. minimus* (Bl.) J. J. Sm., of Java, and the Fijian endemic *C. godeffroyanus* (Reichb. f.) L. O. Williams. This peculiar disjunct distribution indicates that the generic disposition of species from the intervening regions should be checked; a somewhat similar case is found in the little genus *Calyptosepalum* (cf. Santalaceae).

BALANOPSIDACEAE

Balanops Baill. The single genus of the Balanopsidaceae has a narrow distribution, from Queensland to New Caledonia, the New Hebrides, and

[2] I am greatly indebted to Dr. Richard E. Schultes and Mr. Charles Schweinfurth, of the Ames Orchid Herbarium, Harvard University, for checking these records of Orchidaceae.

Fiji. The variability of the one Fijian species, *B. vitiensis* (A. C. Sm.) Hjelmqvist, was discussed by me in Jour. Arnold Arb. **31**: 149–150. 1950.

PROTEACEAE

Kermadecia Brongn. & Gris. This small genus has a very compact range, with seven species in New Caledonia (Guillaumin, Fl. Nouv.-Caléd. 108. 1948), one in Queensland, one in the New Hebrides, and two endemic Fijian species, *K. vitiensis* Turrill and *K. ferruginea* A. C. Sm., terminating its range to the east.

SANTALACEAE

Calyptosepalum S. Moore. That *Calyptosepalum* does not belong in the Santalaceae, where it was placed by Moore, has been pointed out by I. W. Bailey and A. C. Smith (in Jour. Arnold Arb. **34**: 52–64. 1953); we suspect it to represent an independent family, as yet unproposed, but for purposes of the present mention I list it where originally described. The distribution of the two known species of *Calyptosepalum* raises many questions. The original Sumatran species, *C. sumatranum* S. Moore, appears to be very rare, and the second species, *C. pacificum* I. W. Bailey & A. C. Sm., is a Fijian endemic known only from the type collection.

AMARANTHACEAE

Deeringia R. Br. The genus reaches its southeastern limit in Fiji, where it is represented by *D. amaranthoides* (Lam.) Merr., widely distributed from continental Asia through Malaysia and Australia to Micronesia and Fiji.

RANUNCULACEAE

Clematis L. This large and widespread genus, probably with more than 200 species, occurs in Australasian regions as far east as New Caledonia and Fiji, but I find no records of it in Polynesia. Kuntze (in Verh. Bot. Ver. Brand. **26**: 83–202. 1885) does not mention any distribution of the genus beyond Fiji in the Pacific. The Fijian species, *C. pickeringii* A. Gray, has been attributed a range including New Caledonia, New Guinea, and even Australia by some authors; Kuntze treats this taxon as *C. aristata* var. *pickeringii* (A. Gray) Kuntze.

MENISPERMACEAE

Pachygone Miers. Diels (in Pflanzenr. **46** [IV. 94]: 241–246. 1910) recognizes 11 species of *Pachygone*, but several have since been added. The genus occurs from India and southern China to Micronesia, New Guinea, northern Australia, and New Caledonia, with a single terminating endemic species, *P. vitiensis* Diels, in Fiji. I find no report of the genus from the Solomons or the New Hebrides.

DEGENERIACEAE

Degeneria I. W. Bailey & A. C. Sm. This monotypic genus, the basis of an endemic family related to the Magnoliaceae and Himantandraceae,

has been discussed at some length (in Jour. Arnold Arb. **23**: 356–365. 1942, **30**: 1–38. 1949).

ANNONACEAE

Desmos Lour. This essentially Indo-Malaysian genus is known in Fiji from *D. insularis* and *D. leucanthus*, endemic species described by the writer, although otherwise it appears not to have been reported east of the Philippines.

Polyalthia Bl. Nine endemic Fijian species mark the eastern boundary of this large genus, which ranges from Africa and Asia to Australia and Melanesia. It should be noted, however, that the probable occurrence of the genus in Tonga is indicated by one of Professor T. G. Yuncker's recent collections, although works on the plants of Tonga do not list it. This would be an expected continuation of the range, especially since one of the Fijian species is known from southern Lau.

Xylopia L. This large genus, occurring in tropical regions in America, Africa, and Asia, extends in Melanesia as far as Fiji, where three endemic species described by the writer terminate its range to the east.

Cyathocalyx Champion. Four endemic Fijian species described by me (cf. Jour. Arnold Arb. **31**: 160–164. 1950) mark the eastern boundary of this Indo-Malaysian genus.

Richella A. Gray. This is apparently the correct generic name for the large genus currently passing as *Oxymitra* Hook. f. & Thoms. Its extensive Indo-Malaysian and African distribution extends in Melanesia to New Caledonia and Fiji, in which latter group it ends in the endemic *R. monosperma* A. Gray, the type of the genus. The 1954 International Congress did not accept my proposal to conserve *Oxymitra* (1855) in the sense of the annonaceous genus (cf. Taxon 3: 115. 1954), and so *Richella* (1852) must be used as the correct name for this concept, if the view prevails that the two taxa are congeneric (cf. A. C. Sm. in Bishop Mus. Bull. **141**: 62. 1936). In this case the name *Friesodielsia* van Steenis (in Bull. Bot. Gard. Buitenz. III. **17**: 458. 1948) will not be necessary and approximately 80 new combinations under *Richella*, thus far applied only to the Fijian species, will be needed.

CUNONIACEAE

Geissois Labill. The range of *Geissois* centers in New Caledonia, but some of its approximately 17 species occur in Australia, the New Hebrides, and Fiji. In the latter archipelago four endemic species are known (cf. A. C. Sm. in Jour. Arnold Arb. **33**: 120–128. 1952).

Pullea Schlechter. Eight species are known in *Pullea*, six of them in New Guinea (cf. Perry in Jour. Arnold Arb. **30**: 163–165. 1949), one in Queensland, and one, *P. perryana* A. C. Sm. (in op. cit. **33**: 148. 1952), in Fiji. The genus is as yet unreported from the Solomons or the New Hebrides.

CONNARACEAE

Connarus L. *Connarus* has a tricentric distribution in America, Africa, and Asia, from which it extends eastward through Malaysia as far as Fiji; here it ends in the endemic *C. pickeringii* A. Gray.

LEGUMINOSAE

Parkia R. Br. *Parkia*, a genus of 30 or more species, occurs in tropical America, Africa, and the southeastern Asia–Malaysia region. Although a species is recorded from Micronesia, there is a wide distributional gap east of the large Malaysian islands to Fiji, where *P. parrii* Horne ex Baker terminates its range.

Cynometra L. The 60 or more species of *Cynometra* are found in tropical America and Africa, as well as in southeastern Asia, Malaysia, and Micronesia. One species is recorded from New Caledonia and two from Fiji, *C. falcata* A. Gray and *C. insularis* A. C. Sm., terminating the generic range.

Kingiodendron Harms. The very scattered distribution of the four species of *Kingiodendron* has been discussed by Burtt (in Kew Bull. **1936**: 461. 1936). The species, of very local range, occur in southern India, the Philippines and New Guinea (cf. Merrill & Perry in Jour. Arnold Arb. **23**: 399. 1942), the Solomon Islands, and Fiji, where *K. platycarpum* Burtt is the most easterly species.

Storckiella Seem. This little genus is represented by two New Caledonian species and by *S. vitiensis* Seem. The last has been considered endemic to Fiji, but Guillaumin (Fl. Nouv.-Caléd. 156. 1948) lists it from New Caledonia, without documentation.

LINACEAE

Durandea Planch. This genus of 13 species, according to Stapf (in Hook. Ic. Pl. **29**: *tab. 2822*. 1906), has a range of Borneo, New Guinea, and Queensland to the Solomon Islands and New Caledonia, with a single outlying endemic species, *D. vitiensis* Stapf, in Fiji.

RUTACEAE

Wenzelia Merr. In his treatment of the subfamily Aurantioideae, Swingle (in Webber & Batchelor, Citrus Industry **1**: 129–474. 1943) discusses *Wenzelia* as having nine species, distributed 1 in the Philippines, 6 in New Guinea, 1 in the Solomon Islands, and 1 in Fiji. The endemic Fijian species, *W. kambarae* Swingle, is known only from the Lau group.

SIMAROUBACEAE

Amaroria A. Gray. This endemic monotypic genus is known only from *A. soulameoides* A. Gray, one of the most abundant small trees in the Fijian forests. The genus is placed by Engler (in E. & P. Nat. Pfl. ed. 2. **19a**: 393–394. 1931) as one of three genera in the tribe Soulameae.

Engler (loc. cit.) also mentions the related genus *Soulamea* Lam. as occurring in Fiji; its stated range extends from the Seychelles through Malaysia to Micronesia, New Guinea, New Caledonia, and Fiji, where the widespread *S. amara* Lam. is said to occur. I have seen no other reports of the occurrence of *Soulamea* in Fiji, nor are any herbarium records available to me. Therefore, until this occurrence is further verified, I think that a misidentification was probable. The two species concerned are superficially rather similar, but differences in flowers and fruits, as outlined by Engler, appear to be of generic significance.

Another simaroubaceous genus indicated as terminating in Fiji is *Brucea* J. F. Mill., this record being based on *B. quercifolia* Seem., a supposed endemic. This species is not mentioned by Engler (in op. cit. 386–387. 1931), who indicates the generic range as from tropical Africa through Malaysia and the Philippines to Australia. Without having seen Seemann's type, I am inclined to believe that he had a specimen of *B. javanica* (L.) Merr. (in Jour. Arnold Arb. **9**: 3. 1928, a binomial ignored by Engler); both Gillespie and I have collected this species on Taveuni, where it is either cultivated or apparently naturalized. *Brucea,* therefore, may quite confidently be excluded from the indigenous flora of Fiji.

MALPIGHIACEAE

Hiptage Gaertn. Niedenzu (in Pflanzenr. **91** [IV. 141]: 67–84. 1928) recognizes 22 species of *Hiptage*, in addition to four uncertain species, among which is the Fijian *H. myrtifolia* A. Gray. Several species have since been added, the range of the genus being from India, southern China, and Formosa to the Malaysian islands and Fiji, although the outlying *H. myrtifolia* (see Jour. Arnold Arb. **31**: 288. 1950, for distribution) is far removed from the remaining species.

EUPHORBIACEAE

Drypetes Vahl. Widespread in the tropics of both hemispheres and represented by at least 160 species (Pax & Hoffm. in E. & P. Nat. Pfl. ed. 2. **19c**: 72–73. 1931), *Drypetes* extends into Malaysia and Micronesia, terminating to the southeast in Fiji. Here it is represented by *D. vitiensis* Croizat (in Sargentia **1**: 49. 1942), an endemic species which was not further related by its author.

Buraeavia Baill. This small genus is known only from two New Caledonian species and the Fijian endemic *B. horneana* A. C. Sm. (in Jour. Arnold Arb. **33**: 374. 1952). Pax & Hoffmann (in op. cit. 75) treat *Buraeavia* as a synonym of *Longetia* Baill., which genus extends westward to the Malay Peninsula but remains very small, with six species according to the Pax & Hoffmann treatment.

Cleistanthus Hook. f. According to Jablonszky (in Pflanzenr. **65** [IV. 147. VIII]: 8–54. 1915), *Cleistanthus* includes at least 106 species and has a range from Africa to Malaysia, Australia, New Caledonia, and Micronesia. In describing the Fijian endemic *C. micranthus*, Croizat ex-

tended the known range to the east and placed his species in the small section *Australes* Jabl., with species only in Queensland and New Caledonia.

Cleidion Bl. A genus of about 20 species, *Cleidion* occurs in tropical America, Africa, and southeastern Asia, with an extension eastward to Micronesia, New Caledonia, and Fiji. In Fiji it is represented by the endemic *C. leptostachyum* (Muell. Arg.) Pax & Hoffm.

Endospermum Benth. *Endospermum*, with at least 15 or 20 species, ranges from southeastern Asia to New Guinea, except for two endemic species in Fiji, *E. macrophyllum* (Muell. Arg.) Pax & Hoffm. and *E. robbieanum* A. C. Sm. Records of the genus in the Solomons, the New Hebrides, or New Caledonia appear to be lacking.

Stillingia Garden ex L. Pax & Hoffmann (in E. & P. Nat. Pfl. ed. 2. **19c**: 198. 1931) mention *Stillingia* as including about 30 species, while Rogers (in Ann. Mo. Bot. Gard. **38**: 207–259. 1951) recognizes 23 American species. Elsewhere, the genus occurs in Madagascar and Mauritius and has one endemic Fijian species, *S. pacifica* Muell. Arg. This extraordinary generic distribution has been questioned by various students but apparently receives morphological support. Neither the mentioned authors nor Croizat (in Bishop Mus. Occ. Pap. **18**: 71. 1944) find any grounds for removal of the Fijian species from the genus.

ANACARDIACEAE

Semecarpus L. f. The genus ranges from continental Asia through Malaysia and into Micronesia, Australia, New Caledonia, the New Hebrides and Fiji, where is is terminated by the endemic *S. vitiensis* (A. Gray) Engl.

CELASTRACEAE

Elaeodendron Jacq. The Indo-Malaysian portion of the range of *Elaeodendron* extends from southeastern Asia and Australia to New Caledonia, the New Hebrides, and Fiji, where it is terminated by the endemic *E. vitiense* A. C. Sm.

HIPPOCRATEACEAE

Salacia L. The genus *Salacia* has about 30 species in America and perhaps as many or more in the Asiatic tropics, where it extends eastward to Micronesia, the New Hebrides, New Caledonia, and Fiji. In the last archipelago it is represented by two endemic species described by me, *S. vitiensis* and *S. pachycarpa*.

SAPINDACEAE

The two genera of Sapindaceae with ranges terminating in Fiji (cf. A. C. Sm. in Jour. Arnold Arb. **31**: 299–302. 1950) are remarkable for their disjunct distributions.

Koelreuteria Laxm. This sharply marked genus, with eight species, is limited to eastern Asia and Formosa with the exception of the Fijian

endemic, *K. elegans* (Seem.) A. C. Sm. (in Contr. U. S. Nat. Herb. **30**: 518. 1952). Its absence from the intervening areas is puzzling. A somewhat similar, but more extended, type of distribution is noted in *Rhamnella* (Rhamnaceae).

Cossignia Commers. In a different way, the distribution of *Cossignia* is just as puzzling as that of *Koelreuteria*; it is known from four species, of which two are from the Mascarene Islands and one from New Caledonia, the fourth being the recently described Fijian endemic, *C. pacifica* A. C. Sm.

RHAMNACEAE

Ventilago Gaertn. According to Suessenguth (in E. & P. Nat. Pfl. ed. 2. **20d**: 151. 1953), *Ventilago*, with about 40 species, ranges from Africa and Madagascar through the Indo-Malaysian region as far east as Micronesia, New Caledonia, and Fiji. In the last archipelago it is represented by the endemic *V. vitiensis* A. Gray.

Smythea Seem. Suessenguth (op. cit. 154) mentions the genus as having about seven species and a range from southeastern Asia through Malaysia to Micronesia, New Guinea, and Fiji; I have found no records of its occurrence farther east. To the species occurring in Fiji, *S. lanceata* (Tul.) Summerhayes, Suessenguth attributes a very wide distribution extending to Micronesia and into Mayalsia as far as Borneo and the Philippines.

The remaining genera of Rhamnaceae that occur in Fiji have ranges extending farther east, but mention should be made of *Dallachya* F. Muell., which Suessenguth (op. cit. 149) maintains as a monotypic genus with a range from Queensland to Fiji. He appears to have overlooked, or to have disagreed with, my reduction of *Dallachya* to *Rhamnella* (in Bull. Torrey Club **70**: 544. 1943; cf. also Guillaumin, Fl. Nouv.-Caléd. 203. 1948). At any rate, the species concerned, *Rhamnella vitiensis* (Benth.) A. C. Sm., ranges from Queensland and New Guinea through Fiji to Tonga.

VITACEAE

Cayratia Juss. In his recent treatment, Suessenguth (in E. & P. Nat. Pfl. ed. 2. **20d**: 277–282. 1953) accepts about 45 species of *Cayratia*, which is distributed from Africa and southeastern Asia, including Japan, through Malaysia to Micronesia, the Solomon Islands, Australia, New Caledonia, and Fiji. In the last archipelago it is represented by two endemic species, *C. seemanniana* A. C. Sm. and *C. acuminata* (A. Gray) A. C. Sm. (in Sargentia **1**: 55–57. 1942). Apparently Suessenguth overlooked my binomials, for he again makes the combination *C. acuminata* and takes the Australian *C. saponaria* (Benth.) Seem. in a very inclusive sense.

Tetrastigma Planch. Suessenguth (in op. cit. 318–329. 1953) mentions *Tetrastigma* as having about 93 species and a range from southeastern Asia through Malaysia to New Guinea and Australia. The genus also occurs in the Solomon Islands (Merr. & Perry in Jour. Arnold Arb. **22**: 376. 1941)

and Fiji, where it is represented by the endemic *T. vitiensis* (A. Gray) A. C. Sm. (in Bishop Mus. Bull. **141**: 92. 1936). Apparently Suessenguth disagrees with this disposition of *Cissus vitiensis* A. Gray, if this is the implied basionym of his combination *Cayratia vitiensis* (A. Gray) Suesseng. (in op. cit. 281. 1953). Neither this combination nor the above-mentioned *C. acuminata* are validly published by Suessenguth in accordance with Article 42 of the International Code (1952).

TILIACEAE

Berrya Roxb. Burret (in Notizbl. Bot. Gart. Berlin **9**: 605–607. 1926) recognizes three species of *Berrya*, extending from southeastern Asia into Malaysia. To these should now be added a New Guinean species (Merr. & Perry in Jour. Arnold Arb. **20**: 339. 1939) and the outlying Fijian endemic, *B. pacifica* A. C. Sm. (in Jour. Arnold Arb. **31**: 303. 1950).

Microcos L. *Microcos*, a genus of about 75 species, occurs in Africa and more extensively from southeastern Asia into Malaysia as far as New Guinea (cf. Burret in op. cit. 756–796. 1926). A single outlying endemic, *M. vitiensis* A. C. Sm., terminates the range in Fiji, but I find no records of the genus in the New Hebrides or Solomon Islands.

STERCULIACEAE

Pimia Seem. This genus, well enough described and illustrated by Seemann (Fl. Vit. 25. *pl. 5.* 1865) is too little known for proper evaluation, although such students as Bentham & Hooker and Schumann (in Engler & Prantl) have accepted it as an independent, endemic, monotypic genus. The single species, *P. rhamnoides* Seem., may be known only from the type collection; at least I find no other records in the literature or in herbaria.

Firmiana Marsigli. *Firmiana*, with about 20 known species, has a distribution extending from Africa (west tropical Africa, Tanganyika) to eastern Asia, the Philippines, and Malaysia, where it extends to New Guinea. An outlying Fijian endemic, *F. diversifolia* A. Gray, which is locally quite frequent, terminates the generic range.

Pterocymbium R. Br. This small genus ranges from southeastern Asia into Malaysia; Kostermans (in Reinwardtia **1**: 41–49. 1950) has recently discussed the three species occurring in Malaysia. The only record of the genus east of New Guinea and the Bismarck Archipelago is that of the endemic Fijian *P. oceanicum* A. C. Sm. (in Jour. Arnold Arb. **27**: 320. 1946).

DILLENIACEAE

Hibbertia Andr. This large genus of at least 110 species (Gilg & Werdermann in E. &. P. Nat. Pfl. ed. 2. **21**: 21–30. 1925) occurs abundantly in Australia and New Caledonia, with two species in New Guinea (cf. A. C. Sm. in Jour. Arnold Arb. **22**: 497. 1941) and one in Fiji. I believe the Fijian plant to be indistinguishable from the New Caledonian *H. lucens* Brongn. & Gris.

Dillenia L. According to the recent comprehensive monograph by Hoogland (in Blumea 7: 1–145. 1952), *Dillenia* (including *Wormia* Rottb.) occurs from Madagascar through southern Asia, Malaysia, and Queensland to Fiji; 55 species are recognized by him. The single Fijian species, *D. biflora* (A. Gray) Martelli, is locally very abundant, and Hoogland believes that it also occurs in the New Hebrides, where Guillaumin recognized his *D. neoebudica* as a distinct species.

SAURAUIACEAE

Saurauia Willd. This very large genus (at least 250 species according to Gilg & Werdermann in E. & P. Nat. Pfl. ed. 2. 21: 42–45. 1925) is widely distributed in tropical America and the Old World, where it occurs from southeastern Asia through Malaysia to northern Australia and the Solomon Islands, with one locally abundant endemic, *S. rubicunda* (A. Gray) Seem., in Fiji. I find no record of the genus in the New Hebrides, and Guillaumin (Fl. Nouv.-Caléd. 1948) does not list it from New Caledonia.

OCHNACEAE

Brackenridgea A. Gray. This genus, erected for a single Fijian species, *B. nitida* A. Gray, is now known to occur also in Africa and from the Malay Peninsula to New Guinea and Queensland (cf. Gilg in E. & P. Nat. Pfl. ed. 2. 21: 74–75. 1925). Although the Fijian endemic is quite common locally, the genus is unrecorded from the intervening archipelagos as far as New Guinea.

ELATINACEAE

Elatine L. This cosmopolitan genus is supposedly known in the Pacific region, other than New Zealand, only from the Fijian record based on two collections and believed (cf. Jour. Arnold Arb. **26**: 101. 1945) to represent *E. gratioloides* A. Cunn., otherwise known from Australia and New Zealand.

VIOLACEAE

Rinorea Aubl. Melchior (in E. & P. Nat. Pfl. ed. 2. **21**: 349–352. 1925) recognizes about 260 species of *Rinorea*, but many others have been more recently described. The genus is widespread in America and Africa, and in the Indo-Malaysian region it extends from southeastern Asia to Micronesia, New Guinea, the Solomon Islands, and Fiji, where the endemic *R. storckii* (Seem.) Melchior terminates its range. *Rinorea* seems to be absent from Australia and New Caledonia.

Agatea A. Gray. *Agatea* (Melchior in op. cit. 360–361. 1925) has a compact range in New Guinea, the Solomon Islands (Merr. & Perry in Jour. Arnold Arb. **24**: 209. 1943), New Caledonia, and Fiji, where its distribution ends in the endemic *A. violaris* A. Gray. There are about 13 species in the genus.

BEGONIACEAE

Begonia L. This widespread genus appears not to be indigenous in the Pacific east of Fiji, where it is represented by the endemic *B. vitiensis* A. C. Sm. The genus has been listed from the New Hebrides without mention of a species.

THYMELAEACEAE

Gonystylus Teijsm. & Binn. About 22 species are now believed to represent *Gonystylus*, which has a distribution extending from the Malay Peninsula through the Philippines and New Guinea to the Solomon Islands and Fiji, where the range is ended by the endemic *G. punctatus* A. C. Sm. A key to the species and further comments are provided by Airy Shaw (in Kew Bull. **1947**: 9–16. 1947, **1950**: 138–147. 1950) and C. T. White (in Jour. Arnold Arb. **31**: 96. 1950).

ALANGIACEAE

Alangium Lam. The range of *Alangium* extends from southeastern Asia and Africa to Australia, Malaysia, and Melanesia, where it occurs in the Solomon Islands, New Caledonia, and Fiji. The widespread *A. villosum* (Bl.) Wang. terminates in Fiji with an endemic subspecies, subsp. *vitiense* (A. Gray) Bloemb., according to the comprehensive treatment of Bloembergen in Bull. Jard. Bot. Buitenz. III. **16**: 139–235. 1939.

MYRTACEAE

Cleistocalyx Bl. In their informative revision of *Cleistocalyx*, Merrill & Perry (in Jour. Arnold Arb. **18**: 322–343. 1937) indicate its range as southeastern Asia through Malaysia to northern Australia, New Caledonia, and Fiji; they do not mention any records from the Solomon Islands or the New Hebrides. The five endemic Fijian species, terminating the generic range, are separated from the rest of the genus as section *Acicalyptus* (A. Gray) Merr. & Perry.

Piliocalyx Brongn. & Gris. This supposedly endemic New Caledonian genus, with eight species (according to Guillaumin, Fl. Nouv.-Caléd. 247. 1948), has recently been reported from Fiji by Perry (in Jour. Arnold Arb. 31: 370. 1950), who identifies the Fijian material as *P. wagapensis* Brongn. & Gris, one of the New Caledonian species.

Cloëzia Brongn. & Gris. Ten species of *Cloëzia* (*Mooria* Montr.) are known, eight of them from New Caledonia (cf. Guillaumin, Fl. Nouv.-Caléd. 236. 1948), one from the Philippines (Merr. Enum. Phil. Fl. Pl. 3: 182. 1923), and one from Fiji. The correct name for this concept appears to be *Cloëzia* Brongn. & Gris (1863), because *Mooria* Montr. (in Mém. Acad. Sci. Lyon II. **10**: 207. 1860) is a homonym of *Moorea* Lem. (1854, = *Cortaderia* Stapf, nom. conserv.). The different spelling used by Montrousier might conceivably be taken to indicate that these names are not homonyms, but actually *Mooria* was named after Charles Moore, Director

of the Botanic Garden in Sydney, and therefore is an orthographic variant of *Moorea*, the correct form (cf. Int. Code, Rec. 82B. 1952). Guillaumin, indeed, used the spelling *Moorea* for the myrtaceous genus (loc. cit.). In view of these facts, the endemic Fijian plant should be known as **Cloëzia microphylla** (A. C. Sm.) comb. nov. (*Mooria microphylla* A. C. Sm. in Bishop Mus. Bull. **141**: 110. *fig. 57, e-g.* 1936).

Tristania R. Br. *Tristania*, with approximately 30 species, is distributed from southeastern Asia through Malaysia to New Guinea, Australia, and New Caledonia, and is represented in Fiji by an outlying endemic, *T. vitiensis* A. C. Sm. I find no record of the genus in the Solomon Islands or the New Hebrides.

ARALIACEAE

Plerandra A. Gray. The distribution of *Plerandra* extends westward at least to New Guinea and eastward through the Solomon Islands to Fiji; I do not find it recorded from the New Hebrides. In Fiji there are at least seven endemic species and probably more, as some seem still to be undescribed.

VACCINIACEAE

Paphia Seem. Although apparently only five binominals have thus far been referred to *Paphia*, giving the genus a distribution in New Guinea, Queensland, and Fiji, it is probable that several other Papuasian species described under *Agapetes* (cf. Sleumer in Bot. Jahrb. **70**: 95–106. 1939) belong here. Sleumer's reduction of *Paphia* to *Agapetes* is not accepted by Airy Shaw (in Kew Bull. **1948**: 77–104. 1948), who limits *Agapetes* to 64–67 continental species. In any case, the Fijian *Paphia vitiensis* Seem. represents the eastern limit of the generic concept.

EPACRIDACEAE

Leucopogon R. Br. *Leucopogon*, with many species in Australia and New Caledonia, reaches the eastward extension of its range in Fiji, where it is represented by *L. cymbulae* Labill. This species is fairly frequent locally, as in northern Vanua Levu; it also occurs in New Caledonia and the New Hebrides.

MYRSINACEAE

Tapeinosperma Hook. f. As revised by Mez (in Pflanzenr. 9 [IV 236]: 162–171. 1902), *Tapeinosperma* includes 26 species, but this number has been approximately doubled since then. The genus centers in New Caledonia but ranges from Australia and New Guinea through the New Hebrides to Fiji, where 11 endemic species are known.

SYMPLOCACEAE

Symplocos Jacq. The huge genus *Symplocos*, with several hundred species in America and the Indo-Malaysian region, extends eastward in the Old World to Micronesia, the New Hebrides, New Caledonia, and Fiji.

where its range is terminated by two endemic species, *S. leptophylla* (Brand) Turrill and *S. turrilliana* A. C. Sm.

LOGANIACEAE

Strychnos L. The large and widespread genus *Strychnos*, with many species in America and Africa, has been discussed in its Asiatic range by A. W. Hill (in Kew Bull. **1911**: 281–302. 1911, **1917**: 121–210. 1917). The Indo-Malaysian range of the genus extends to New Guinea and northern Australia, with a single outlying terminating species, *S. vitiensis* A. W. Hill, in Fiji. I have not noted references to the genus in the archipelagos between New Guinea and Fiji.

Couthovia A. Gray. Centering in New Guinea, the range of *Couthovia* extends from the Philippines and Celebes to Micronesia, New Caledonia, the New Hebrides, and Fiji, where six endemic species terminate it (cf. A. C. Sm. in Sargentia **1**: 99–107. 1942).

APOCYNACEAE

Pagiantha Markgraf. As discussed by Markgraf (in Notizbl. Bot. Gart. Berlin **12**: 540–552. 1935), *Pagiantha* has a distribution from continental Asia through the Philippines into Melanesia, terminating in a single endemic Fijian species, *P. koroana* Markgraf. I have already (in Jour. Arnold Arb. **33**: 114. 1952) suggested that his name must fall into the synonymy of *P. thurstonii* (Baker) A. C. Sm.

Carruthersia Seem. This genus extends from the Philippines to the New Hebrides and Fiji, having two endemic species in the latter archipelago, *C. scandens* Seem. and *C. latifolia* Gillespie.

Parsonsia R. Br. As taken to include *Lyonsia* R. Br., *Parsonsia* extends from continental Asia through Malaysia to Australia, New Caledonia, the New Hebrides, and Fiji, where it is represented by the endemic *P. laevis* (A. Gray) Markgraf, from which *P. smithii* Markgraf, in my opinion, is scarcely distinct.

ASCLEPIADACEAE

Gymnema R. Br. This genus occurs from Africa into Malaysia, Australia, New Caledonia, and Fiji, where it is represented by two endemic and rather infrequent species, *G. stenophyllum* A. Gray and *G. subnudum* A. Gray.

VERBENACEAE

Gmelina L. *Gmelina*, with about 34 species, has a range extending from Africa and southeastern Asia through Malaysia to Micronesia, the Solomon Islands, New Caledonia, and Australia, with an outlying endemic species, *G. vitiensis* Seem., in Fiji.

ACANTHACEAE

Dyschoriste Nees. The endemic Fijian representative of this genus, *D. repanda* (A. Gray) A. C. Sm., marks the eastern limit of the Asiatic-

Malaysian portion of the genus, which also has species in America and Africa. The American species have been revised by Kobuski (in Ann. Mo. Bot. Gard. **15**: 9–90. 1928).

RUBIACEAE

The Rubiaceae is not only the largest family of plants in Fiji, from the point of view of number of indigenous species, but it is also the most interesting in its distribution. Here seven genera occur on the eastern border of their ranges, while five others are believed endemic.

Xanthophytum Reinw. ex Bl. This little genus, of approximately twelve species, occurs from southeastern Asia through the larger islands to New Guinea, with one endemic outlying species in Fiji, *X. calycinum* (A. Gray) Benth. & Hook. f. ex Drake. No occurrence of the genus in the New Hebrides or Solomon Islands has been noted.

Lindenia Benth. *Lindenia* has a curious, if not incredible, distribution, being known from Central America, New Caledonia, and Fiji. A distribution of this sort must be checked by a specialist in the family before it can be taken at face value. The American population is generally treated as a single species, occurring with some frequency from Mexico to Panama. *Lindenia vitiensis* Seem., described from Fiji, is certainly infrequent there; it has also been accredited to New Caledonia, although another species has been described from that island. Seemann's note on the occurrence of *L. vitiensis* in Samoa (Fl. Vit. 430. 1873) is based on a Graeffe collection; until this record is further supported one may suspect that Graeffe's specimen was from Fiji, as his labels are not too dependable.

Dolicholobium A. Gray. Like several other genera, *Dolicholobium* was believed endemic to Fiji long after its description, until 1900 in this case. It is now known to consist of about 23 species, distributed from the Philippines (2) through New Guinea (8), the Bismarcks (1), the Solomons (6), and the New Hebrides (1) to Fiji (5). The species are all considered endemic to their respective groups. Fosberg (in Sargentia **1**: 118–119. 1942) suggests that there may be only two species in Fiji, but this disposition needs to be examined.

Sukunia A. C. Sm. The single species referred to this endemic genus, *S. pentagonioides* (Seem.) A. C. Sm. (in Bishop Mus. Bull. **141**: 137. 1936), is rare in Fiji. I consider the closest relationship of the plant to be with the widespread *Randia* L.

Airosperma Lauterb. & K. Schum. A single Fijian endemic, *A. trichotomum* (Gillespie) A. C. Sm., terminates the range of this small genus, which otherwise is known only from four New Guinean species. A discussion of *Airosperma* and *Abramsia* Gillespie was given by me in Jour. Arnold Arb. **26**: 107–108. 1945.

Mastixiodendron Melchior. Like the preceding, *Mastixiodendron* is an essentially New Guinean genus of narrow distribution, with three species occurring from Halmahera to the Solomon Islands (cf. Merr. & Perry in

Jour. Arnold Arb. **26**: 254–256. 1945) and two species endemic to Fiji (cf. A. C. Sm. in op. cit. 108–110. 1945, where the supposedly endemic genus *Dorisia* Gillespie is reduced).

Readea Gillespie. The type-species of this endemic genus, *R. membranacea* Gillespie, is fairly frequent in Fiji; two rarer plants, of which the generic position is not entirely certain, have been placed here by Fosberg (in Sargentia **1**: 136–137. 1942). The general relationship of *Readea* is with *Psychotria* L.

Gillespiea A. C. Sm. Another endemic Fijian genus, monotypic as far as known, is *Gillespiea* (in Bishop Mus. Bull. **141**: 158. 1936), which is also related to *Psychotria*. The single species, *G. speciosa* A. C. Sm., has been found only on Vanua Levu.

Hedstromia A. C. Sm. Like the preceding, *Hedstromia* (op. cit. 146. 1936) is a monotypic endemic, its species, *H. latifolia* A. C. Sm., being known only from Vanua Levu. *Hedstromia* is also referred to the Psychotrieae.

Amaracarpus Bl. Approximately 60 species of *Amaracarpus* have been described, the genus occurring from the Malay Peninsula through Malaysia to Micronesia, New Guinea (42 species), the Solomon Islands (1 species), and Fiji, where the endemic *A. musciferus* A. C. Sm. terminates the range.

Squamellaria Becc. This endemic genus (Beccari, Malesia **2**: 228–230. 1886) is represented by two Fijian species, *S. imberbis* (A. Gray) Becc. and *S. wilsonii* (Horne ex Baker) Becc.

Hydnophytum Jack. Approximately 100 species of *Hydnophytum* have now been described, about 60 of them from New Guinea (cf. Merr. & Perry in Jour. Arnold Arb. **26**: 14–26. 1945). The genus is distributed from southeastern Asia through Malaysia to the Solomon Islands and Fiji, where five endemic species terminate its range.

CUCURBITACEAE

Neoalsomitra Hutchinson. In establishing this genus, Hutchinson (in Ann. Bot. n.s. **6**: 97 seq. 1942) recognizes 22 species, ranging from India to Australia, New Guinea, and Formosa. One species, *N. integrifoliola* (Cogn.) Hutchinson, is also reported from Fiji, on the basis of a specimen collected by Horne on Vanua Levu. The species is not known to me from any other Fijian collection, and I suspect that the record may be discounted on the basis of a misidentification (Horne's specimens often being inadequate) or a mixed label. Until more recent collections indicate the contrary, the range of *Neoalsomitra* may be suspected to terminate in New Guinea.

DISCUSSION

Of the 445 phanerogam genera represented by indigenous species in Fiji (8), 101 have ranges that terminate there. Of these, 13 genera are

TABLE 1

DISTRIBUTION OF PHANEROGAM GENERA WITH RANGES TERMINATING IN FIJI

FAMILY	NO. OF GENERA	WIDE-SPREAD	INDO-MALAYSIAN	AUSTRALIA-N. CALEDONIAN	ENDEMIC
Podocarpaceae	2	1		1	
Araucariaceae	1		1		
Triuridaceae	1		1		
Palmae	8	1	2		5
Orchidaceae	7	1	6		
Balanopsidaceae	1			1	
Proteaceae	1			1	
Santalaceae	1		1		
Amaranthaceae	1		1		
Ranunculaceae	1	1			
Menispermaceae	1		1		
Degeneriaceae	1				1
Annonaceae	5	3	2		
Cunoniaceae	2		1	1	
Connaraceae	1	1			
Leguminosae	4	2	1	1	
Linaceae	1		1		
Rutaceae	1		1		
Simaroubaceae	1				1
Malpighiaceae	1		1		
Euphorbiaceae	6	4	1	1	
Anacardiaceae	1		1		
Celastraceae	1	1			
Hippocrateaceae	1	1			
Sapindaceae	2	1	1		
Rhamnaceae	2	1	1		
Vitaceae	2	1	1		
Tiliaceae	2	1	1		
Sterculiaceae	3	1	1		1
Dilleniaceae	2	1		1	
Saurauiaceae	1	1			
Ochnaceae	1	1			
Elatinaceae	1	1			
Violaceae	2	1	1		
Begoniaceae	1	1			
Thymelaeaceae	1		1		
Alangiaceae	1	1			
Myrtaceae	4		2	2	
Araliaceae	1		1		
Vacciniaceae	1		1		
Epacridaceae	1			1	
Myrsinaceae	1			1	
Symplocaceae	1	1			
Loganiaceae	2	1	1		
Apocynaceae	3		3		
Asclepiadaceae	1	1			
Verbenaceae	1	1			
Acanthaceae	1	1			
Rubiaceae	12	1	6		5
49 Families	101	34	43	11	13

endemic and perhaps not significant in this discussion (although their relationships are without exception toward the west). The remaining 88 genera fall into three general patterns of distribution: 34 of them are widespread, with some species in Africa and/or America as well as in Indo-Malaysian regions; 43 are Indo-Malaysian, although a few extend northwestward as far as China and Japan, while some are limited to the Melanesian archipelagos; and eleven have a predominantly "Australian — New Caledonian" type of distribution. It seems desirable to treat the widespread genera as "terminating" in Fiji, since even when they occur in South America there is no evidence that trans-Pacific migration was involved in the modern distribution; on the other hand, the relationships of the constituent species of these genera indicate that they reached Fiji by an eastward extension of the Indo-Malaysian portion of their ranges. Apparent contradictions of this statement are found in such genera as *Stillingia* (Euphorbiaceae) and *Lindenia* (Rubiaceae), discussed above; but in these cases the disjunction of their ranges is so extraordinary that the need of re-examination of generic lines is indicated.

It is seen, then, that nearly 23 percent of the phanerogam genera with indigenous species in Fiji extend no farther eastward. This figure is perhaps more impressive when one considers that the remaining genera (344 in number) include many common strand plants or plants with wind-carried seeds, to which the over-water distance to Polynesian archipelagos is not a serious obstacle. Most of the genera terminating in Fiji are groups of forest plants, often with heavy seeds, and it may be suspected that land connections were needed to permit their forbears to reach this region. For the most part the Fijian species of these genera are endemic, indicating that the isolation of the archipelago from other remnants of the foundered Melanesian continent has been of considerable duration.

To carry this study to a logical conclusion, similar statistics should be obtained from each archipelago between New Guinea and the American continent. Although this has been done for some island groups in a preliminary way (3), we still lack information to show where in the Pacific the important distributional discontinuities in the ranges of Indo-Malaysian genera occur, and to show also where new elements with relationships to the east become significant. Obviously such data are not obtainable in advance of fairly complete floristic studies, but upon them depends a better comprehension of Pacific phytogeography. It is hoped that this summary will contribute to the final picture; it indicates, I believe, that a sharp distributional break in the continuity of phanerogam genera occurs in or immediately east of Fiji, and it strengthens the premise that this line marks a geological boundary of fundamental significance.

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MITOCHONDRIA AND PRECIPITATES OF A-TYPE VACUOLES IN PLANT CELLS

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With three plates

The ultimate purpose of investigations of cytoplasmic particulates is to determine their function, and this requires the study of living material. The development by Bailey (1) of special techniques for examining living cambial cells, and the study of different properties of vacuoles in relation to staining with vital dyes by Bailey and Zirkle (2) demonstrated the general importance and practicability of living studies in the investigation of protoplasts. The introduction of phase contrast microscopy added another valuable method to such study; and, in combination with vital staining and use of polarized light, made possible the observation and in many cases identification of cytoplasmic components in different types of living cells.

The distinction between mitochondria, plastids, and spherosomes in the epidermal cells of a number of plants has been considered in previous studies [Sorokin (18, 19, 20)]. Present investigations are primarily concerned with the identification of mitochondria and their distinction from precipitates of A-type vacuoles.

Two types of vacuoles are encountered in the living cells of higher plants (Bailey, 1; Bailey and Zirkle, 2; Zirkle, 23). One, the A-type vacuole, becomes intensely magenta or red in aqueous solutions of either Neutral red or Methyl red, while the other, or B-type vacuole, stains orange or reddish-orange in aqueous solutions of Neutral red, but gives no color with Methyl red. A-type vacuoles contain aromatic substances, and tend to form copious dark precipitates with dyes of the type of Neutral red or Janus green B. Because the recognition of mitochondria in living plant cells is based upon their reversible staining with Janus green B, it is important to differentiate them from precipitates of A-type vacuoles when the latter are formed in the cells. It is no less important to distinguish them from the spherosomes (oil globules), which may reflect a delicate green color after the cell preparation has undergone prolonged staining. Hence, the criteria for the recognition of mitochondria are further elaborated here, and to these are added a method for the discrimination of mitochondria and the A-type vacuolar precipitates, using combined vital staining with Janus green B and Neutral red.

Although the ubiquity of mitochondria in living cells is usually taken for granted, there has been no definite demonstration of their permanent

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presence in many plant tissues, including the chloroplast-containing cells of the leaf (Weier, 21; Weier and Stocking, 22). The present study is therefore also concerned with demonstration of ubiquitous occurrence of mitochondria in various chloroplast-containing cells of leaf tissues.

MATERIAL AND METHODS

Because the epidermal and subepidermal cells of lettuce exhibit characteristic A-type vacuoles, while spinach cells show the presence of B-type vacuoles, these plants were selected for this study. Living cells showing cyclosis, or otherwise characterized as being alive by Bailey and Zirkle (2), were considered, only. In the midrib of commercially grown Iceberg lettuce small incisions were made by razor blade. The pieces of epidermis were then stripped off by forceps and floated upon either 10 per cent sucrose (Merck reagent) solution, or 10 per cent commercial sucrose solution. Both double-distilled water and tap water were used. The pH values of the solutions were determined by Beckman's glass electrode pH meter. Similar procedures were followed with strips of epidermis from the petioles of commercially grown spinach. Cross sections from lettuce midribs and spinach petioles were also taken. To insure aerobic conditions during the experiment these sections were made adherent to a piece of wood splinter, which was extended across the flat staining dish, and so were also floated upon the solutions. All solutions were freshly prepared at the beginning of each experiment, and upon addition of sucrose the water was vigorously agitated to incorporate air. Observations were made either immediately after securing the preparations under the phase contrast microscope, or after staining with Janus green B (Hoechst), Neutral red (NACC, NX-6), or a combination of the two dyes. The former was added to the sucrose solutions in dilutions of 1:50,000, while the latter was added to make a dilution of 1:20,000. When used in combination the final solutions of the dyes were mixed together in equal amounts. An average of 45 minutes was allowed for the penetration of the dye, and, with few exceptions, material was examined and photographed during the following hour.

Observations were made under the Zeiss Opton microscope with bright field, phase contrast, and polarization equipment. Neofluar objectives 40/0.75; Ph 40/0.75, Ph 100/1.30, Apochr. 90/1.4 were used with K oculars 8X with magnification changer for factors 1X, 1.6X, and 2.5X. Photomicrographs were made with a 35 mm. camera attachment (R. Winkel G. m. b. H.) Negatives were enlarged as specified.

OBSERVATIONS

The epidermal tissue of lettuce peels off in two layers, the epidermal proper, and the subepidermal. The epidermal cells of the midrib, seen in surface view, have pointed ends, while transverse walls of subepidermal cells are oblique (Hayward, 9). The first material is observed to better

advantage if mounted on the slide with the cutinized side uppermost. Subepidermal cells are better seen when the material is mounted in the reverse way. Both produce an excellent phase contrast when in living condition. In most of the epidermal cells the nucleus is located centrally and is surrounded by a circle of round formed plastids, which are predominantly arranged in a plane parallel to the surface of the cell (Pl. I, fig. 2, fig. 4). However, individual plastids may occur in small numbers at different planes (Pl. I, fig. 1). Although the lettuce tissue is almost etiolated, the round plastids may show a slight green coloring. There is a constant motion of cytoplasm in the vicinity of the nucleus, and the streams circulate the particles through trabeculae towards the peripheral layers of cytoplasm. The most conspicuous particles, easily recognizable under phase contrast, are the spherosomes (Pl. I, figs. 1 and 4). The spherosomes move faster than any other component of the cytoplasm, are highly refractive, and when observed under oil immersion appear as plates with rounded corners which revolve about their axis and continually change the phase contrast from dark to light. Similar behavior was observed in spherosomes of other plant cells (Sorokin, 20). Besides the spherosomes there are other components which can be observed under phase contrast, but cannot be identified without application of vital staining. These are the mitochondria and colorless plastids. The existence of polymorphic colorless plastids in the epidermal, subepidermal, and parenchyma cells of lettuce can be established by exposing head lettuce material to sunlight for 10 or more days in a moist chamber. After this period the exposed material becomes quite green; and numerous polymorphic green plastids of various sizes, including small granules, dumb-bells, and threadlike forms, can be observed in the cells. The existence of mitochondria in the epidermal and subepidermal cells can be tested by the reaction of such particulates to vital staining with Janus green B. In Pl. I, fig. 2 mitochondria are stained blue and appear in the layer of cytoplasm furthermost from the cutinized cell wall. This material was mounted cutinized wall down and was photographed through the subepidermal cell, the outlines of which are visible in the photograph. Similarly, blue stained mitochondria may be seen in the photograph of a subepidermal cell (Pl. I, fig. 3). In both cases mitochondria are quite similar in shape and in about equal number. Besides the spherosomes the other particulate which does not stain with Janus green B after about 45 minutes is the colorless polymorphic plastids. They appear in varying numbers in different cells, and are prominent in the vicinity of the nucleus (Pl. I, fig. 1) and in the parietal layers of the cytoplasm, including that layer next to the cutinized cell wall from which the majority of mitochondria are excluded. That the polymorphic colorless plastids of lettuce may have sizes and shapes very similar to those of mitochondria was observed before (Sorokin, 19). Present photographs lack color distinction, but in Pl. I, fig. 5 it is still possible to see darker forms, mitochondria stained with Janus green B, and lighter forms, unstained plastids.

In cells stained with Janus green B a precipitate begins to form in the

vacuole after a varying period of time, from 1–2 hours. Discrete small granules at first begin to join others to form larger bodies, and at certain stages the staining reaction is so confusing that only an experienced observer can pick out the mitochondria in the preparation. In PL. II, FIGS. 6 and 7 the same cell is pictured at different foci. There vacuolar precipitates are beginning to form in several portions of the vacuole; and although the mitochondria are still colored, they are not clearly distinguished. However, staining with combined Janus green B and Neutral red will precipitate the heavy colloidal substance as a dark cherry-red deposit, while the mitochondria will remain blue. With the passage of time the precipitate increases in amount, to become copious. In PL. II, FIG. 8 the precipitate was dark red, while blue mitochondria could also be discerned. The chloroplasts retained their position in a circle about the nucleus. In PL. II, FIG. 9 a similar precipitate was found in guard cells and adjacent cells. Chloroplasts, mitochondria, and spherosomes were also observed in guard cells. In subepidermal cells the precipitate was finer in structure, copious, and more of an orange-red tint. Janus green B alone will produce in subepidermal cells a precipitate, similar in grain, but of brilliant blue color which refracts green (PL. II, FIG. 10). In epidermal cells Janus green B alone will not produce the heavy clumps typical for those with Neutral red or combination staining. Instead, isolated blue granules and smaller clumps become visible there. Thus, cross sections of the leaf midrib stained with the combined stains reveal precipitates of A-type vacuoles in both epidermal and subepidermal cells.

The cells of parenchyma tissue may show a diffuse reddish coloring with Neutral red or combination staining; but, with very few exceptions, fail to show typical precipitates. The latter occurs, however, in the cells of the bundle sheaths enclosing the vascular bundles.

The character of the precipitate in cells of the bundle sheaths is reminiscent of that found in subepidermal cells in that it is finer grained and lighter red than that of epidermal cells.

Material treated for 24 hours with ferrous chloride solution, one of the tests for tannins, showed a blue-black deposit in epidermal cells, but none in subepidermal or bundle sheath cells. It would seem that substances precipitated by the stains in these differing cells are not the same.

The presence of mitochondria, spherosomes, and plastids were observed in all cells in the cross sections of lettuce midrib studied.

Experiments with spinach were carried out simultaneously with those on lettuce. Pieces of tissue peeled from the petioles of leaves contain epidermal cells with attached subepidermal tissue. In spinach the layer of subepidermal cells found in lettuce is lacking; and the collenchyma tissue does not form a complete cylinder of even thickness immediately under the epidermis, but is more developed in some parts of the petiole, compared with other parts of the same petiole, so as to form little ridges. This condition is similar to that found in the petiole of the beet (Hayward, 9). Therefore, peelings of epidermis may have attached either collenchyma cells or the chlorophyll-bearing parenchyma. Chloroplasts of the latter

may completely fill the cells, or may occur in fewer numbers, to render them suitable for living observations.

The phase contrast optics do not produce such excellent images in spinach as they do in lettuce, but are helpful in the identification of some of their components. PLATE III, FIGS. 11 and 12 show the same epidermal cell stained with a combination of Janus green B and Neutral red and photographed with light and phase contrast objectives of the same magnification. In both cases the chloroplasts and the mitochrondria are clearly identifiable. In PL. III, FIG. 11 mitochondria were stained medium blue, the chloroplasts green, and the vacuolar precipitate a bright red. Under phase contrast, PL. III, FIG. 12, the mitochondrial color was lost, and they appeared as black spherical bodies. The chloroplasts showed more structural details, but the vacuolar precipitate had lost some of the sharpness of outline seen in the light microscope. The spherosomes are difficult to photograph in spinach, due to their small size and rapid motion. They are best observed in cells with pronounced cytoplasmic streaming and in the trabeculae which cross the vacuoles. In PL. III, FIG. 13 a large chlorophyll-bearing parenchyma cell stained with Janus green B was photographed under the light microscope. The preparation had been mounted cuticle side down. The distribution of chloroplasts and the stained mitochondria was uniform throughout the cell. PL. III, FIG. 14 is a photograph of a section of a similar cell at high magnification. Again, the mitochondria were distinct and colored and opaque medium blue at the moment recorded.

In cross sections of the petiole the chlorophyll-bearing parenchyma may occupy from one to several layers, gradually merging with the large cells of the ground parenchyma of the petiole. These large cells show a remarkable development of cytoplasmic trabeculae, which not only cross the vacuole, but form a complex three-dimensional net in which the nucleus is suspended. The chloroplasts are much smaller there than in the chlorophyll-bearing parenchyma; and, together with mitochondria and spherosomes, move about in the cytoplasmic strands. Such a cell, stained with combined Janus green B and Neutral red, showed blue colored mitochondria after one hour and thirty minutes' observation under the microscope. Later the mitochondria lost their color, but the cell remained alive and was observed under the microscope for eight hours. During these observations the trabeculae changed their lengths; and the bulk of the cytoplasm with the suspended nucleus altered its position, gradually moving from one side of the cell to the other.

Staining with the combination of Janus green B and Neutral red gave no A-type vacuolar precipitates in the epidermal and chlorophyll-bearing parenchyma cells of spinach. The guard cells showed magenta colored vacuoles, but unlike those in lettuce did not form precipitates. Precipitates of any colored type were conspicuously absent from the cells of the bundle sheath; yet, they contained chloroplasts, mitochondria, and spherosomes. However, epidermal cells, particularly those in the neighborhood of the stomata, evinced globular red-colored precipitates, such as are shown in PL. III, FIG. 11. This type of precipitate, formed close to the surface of

the vacuole, is characteristic of B-type vacuoles (Bailey and Zirkle, 2; Zirkle, 23).

During staining experiments it was noticed that the color of the combination stain in sucrose solution differed with use of tap or double-distilled water, being brown-violet in the former and amethyst in the latter. To determine the pH values of solutions used and their effects upon staining of mitochondria and upon formation of precipitates in the vacuoles, tests were made and are summarized in TABLE I.

The brand NX–6 of Neutral red in tap water (pH 7.2) and dilution 1:20,000 was red-orange (pH 6.7). In double-distilled water (pH 6.0).

TABLE I

Source of water & dyes	pH	Color	Lettuce Mit.	Lettuce A-type	Lettuce B-type	Spinach Mit.	Spinach A-type	Spinach B-type
Win.tap *	7.2							
Win.tap JgB	6.9	Blue	X	X	..	XXX	..	..
Win.tap Nr	6.7	Red-orange	..	XXX	..	..	..	X
Win.tap JgB Nr	6.85	Brown-violet	X	XXX	..	XXX	..	X
Win.tap 10% com. suc.	7.1							
Win.tap 10% com. suc. JgB	7.0	Blue	X	XXX	..	XXX		
Win.tap 10% com. suc. Nr	6.65	Red-orange	..	XXX	..			X
Win.tap 10% com. suc. JgBNr	6.8	Brown-violet	X	XXX	..	XXX	.	X
Win.tap 10% re. suc.	6.7							
Win.tap 10% re. suc. JgB	6.7	Blue	X	XXX	..	XXX		.
Win.tap 10% re. suc. Nr	6.5	Red-orange	..	XXX	..	..	..	X
Win.tap 10% re. suc. JgBNr	6.6	Brown-violet	X	XXX	..	XXX	..	X
D.dis.	6.0							
D.dis.JgB	5.25	Blue	X	X	..	XXX	..	..
D.dis.Nr	5.05	Cherry-red	..	XXX	..	..	..	..
D.dis.JgB Nr	5.175	Amethyst	X	XX	..	XXX	..	X
D.dis. 10% com. suc.	5.8							
D.dis. 10% com. suc. JgB	5.7	Blue	XXX	XXX	..	XXX	..	..
D.dis. 10% com. suc. Nr	5.4	Cherry-red	..	XXX	.	.	.	..
D.dis. 10% com. suc. JgBNr	5.5	Amethyst	XXX	XXX	..	XXX	.	..
D.dis. 10% re. suc.	5.7							
D.dis. 10% re. suc. JgB	5.65	Blue	XX	XX	..	XXX	..	..
D.dis. 10% re. suc. Nr	5.15	Cherry-red	..	XXX	..	..	..	X
D.dis. 10% re. suc. JgBNr	5.3	Amethyst	XX	XXX	..	XXX	.	..

* The abbreviations stand for following: Win. = Winchester; JgB = Janus green B; Nr = Neutral red; com. suc. = commercial sucrose; re. suc. = reagent sucrose; D.dis. = double distilled; Mit. = mitochondria; XXX = excellent; XX = good; X = average staining.

similar dilution, the color was cherry-red (pH 5.05). Addition of 10 per cent sucrose tended to lower the pH values in all solutions investigated. Reagent grade sucrose lowered the values more than did commercial sugar. There was no appreciable difference in the color of solutions after addition of sucrose. The color of Janus green B remained blue regardless of the pH value of the solutions. Indeed, this was indicated for a wider range (pH 3 to pH 9) by Drawert (8). The addition of Janus green B to the solutions lowered the pH value, though to a lesser degree than the addition of Neutral red. With combination of the dyes, dilutions of 1:50,000 of the green and 1:20,000 of the red being represented in the final mixture, the solutions ranged in color from violet-brown in the higher pH ranges to amethyst in the more acidic pH values. Similarly, the pH readings of the mixed dye solutions represented mean values of the readings of the separate dye-sucrose solutions.

In determining the color of vacuoles after staining with Neutral red or the combination of dyes mentioned previously, it is essential to keep in mind that variations in intensity can be attributed to varying concentrations of the dyes in different vacuoles, to "salt" and "protein" error, etc., (Bailey, 1). In the present study, however, attention was less directed to diffuse coloring of vacuoles than to the formation of colored precipitates. Accordingly, the indications of intensity noted in the columns, "A-type" and "B-type," of TABLE I refer to the precipitate rather than to the vacuole.

The A-type precipitate in lettuce material was very good in virtually all solutions used. Globular precipitates characteristic of B-type vacuoles were observed in spinach after staining with Neutral red or with the combination stain. They were not seen when Janus green B was used alone.

The staining of lettuce mitochondria was only moderately successful in tap water with either type of sucrose used. Better results were obtained when solutions were made up in double-distilled water. Beautifully colored mitochondria were observed in all spinach cells investigated, regardless of the source of water, or of the type of sucrose used.

DISCUSSION

Cell granules and filaments which we now call mitochondria were first stained in the animal cell by Michaelis (15) in 1900. He indicated that the diethyl derivative of Janus green B was essential for the staining reaction, and that the reaction was aerobic. He advised that the procedure be carried out in flat shallow dishes. Other investigators could not repeat the reaction until Bensley (3) reintroduced its use. The history of the use of Janus green B for staining of plant mitochondria began a few years later, but has been running a parallel course. Although mitochondria in higher plants were successfully stained by N. Cowdry (6) and many subsequent observers of the living cell (for literature see Sorokin, 18, 19; Newcomer, 16), the validity and even possibility of the staining procedure is still doubted by some investigators (Newcomer, 16).

According to E. V. Cowdry (5) Janus green B (Hoechst) stains mitochondria specifically; Janus green (Grübler) and Janus green C will not stain them, though these dyes differ only in the substitution of 2 H-atoms for the first, and 2 CH_3-groups for the second, in place of the two C_2H_5-groups. A number of other Janus green B dyes, including Janus green B (Grübler, Berlin West) and Diazingreen Merck were found to stain mitochondria in plants, whereas Janus Green (Grübler, Leipzig) did not (Drawert, 8). Janus green B (diethylsafranine azodimethylaniline chloride) is blue. Upon chemical reduction Janus green B is reduced successively to leuco-Janus green B, to diethylsafranine (red), and to leucosafranine (colorless). Presumably there are several types of leuco-Janus green B (partially reduced forms), and some of them are colorless (Lazarow and Cooperstein, 11). Leuco-Janus green B can be reoxidized to the blue dye by molecular oxygen, or it can be further reduced to the diethylsafranine derivative (red dye) by splitting the azo bond. The second reduction step is not reversible (Lazarow and Cooperstein, 12).

When a mitochondrion is stained blue under aerobic conditions, the Janus green B is in an oxidized state. With the change to anaerobic environmental conditions the dye is reduced to what is presumed to be a colorless leuco-form. The latter may become oxidized again with the return to higher oxygen tensions, restoring the color to the mitochondrion under scrutiny. This property of taking up the colored stain, losing the color, and regaining it again is considered to be a specific reaction for mitochondria. Therefore, in the preceding observations, it is emphasized, only those particulates answering these conditions are here considered to be mitochondria.

The Janus green B reaction in mitochondria is strictly aerobic. This has been continuously reiterated by Bensley (3, 4). In plant cells the reaction was observed only when the pieces of material were floated upon the solution with the dye (Sorokin, 18). To assure the penetration of oxygen in staining the mitochondria from the epidermal cells of onion, Drawert (8) used pure oxygen, which was either supplied beforehand to the solution of the dye on which the strips of epidermis were floating, or was bubbled through it during the staining. In order to stain mitochondria the present author finds it sufficient to aerate the solution by means of a vigorous shaking beforehand.

The rate of penetration of the dye into the cells varies from plant to plant and from cell to cell within the same tissue. As an example, hair cells of *Tradescantia* exhibit cyclosis for hours after mounting the preparation in water with Janus green B; but mitochondrial staining becomes visible only after mechanical pressure is applied to the cover glass. The length of time during which it is possible to observe the colored mitochondria also varies before their decolorization. Drawert (8) reports that he was unable to use a coverglass and had to resort to the use of a water-immersion lens in order to see the mitochondrial reaction.

Michaelis (15) had early pointed out that vital staining does not occur when the respiration rate is very low. The present author (19) could not

obtain the mitochondrial reaction in epidermis explants from iris cultured upon an isotonic sucrose solution for 11 days. The cells were alive, but apparently the rate of respiration was low. In addition, dead cells do not reduce Janus green B, hence, no differential staining takes place (Lazarow and Cooperstein, 11). The preceding points illustrate some reasons why some investigators failed to obtain the mitochondrial reaction in plant cells. Selective staining is specific within a range of precise conditions enumerated above.

Staining of structures other than mitochondria by Janus green B may occur in cells. In dead or dying cells, showing Brownian movement, the cell wall, the cytoplasm, and the nucleus will take up the stain. Besides the colored precipitate of the A-type vacuole already discussed, the spherosomes may also acquire a refractive greenish tint. Drawert (8) has found that the spherosomes of the living onion cell begin to fluoresce green-white or green-yellow under anaerobic conditions after mitochondria stained with Janus green B lose their color. He explained this by postulating the formation of a fluorescent reduced form of Janus green B, which is adsorbed on the spherosomes.

Staining of material containing A-type vacuoles with the combined stains Janus green B and Neutral red distinguishes the vacuolar precipitates from mitochondria by color. This identification of granules occurred in all cases investigated, regardless of the pH value and color of the combined dye solutions. This combination of stains was used by Michaelis (15) for differentiation of cytoplasmic granules. In modern times it is used extensively in haematology for the identification and distinction from mitochondria of other granules in various blood cells (Romeis, 17). The present application of the method to plant material is modified, in that dilute solutions of the dyes are used to assure survival of the cells and aerobic conditions of staining.

The ubiquity of animal cell mitochondria is generally accepted, and it has been demonstrated that the cyclophorase system of enzymes is associated with them (reviewed in De Robertis et al., 7). It is also almost universally agreed that the fundamental properties of animal and plant mitochondria are analogous. Hence, it is logical to assume that the particulate responsible for the respiratory functions must be present in all living cells of plants. The presence of mitochondria in all living epidermal cells studied has been observed by the present author partly in papers cited above, partly in vast unpublished material. It is intended eventually to demonstrate the presence of mitochondria in the cells of different tissues, for which a substantial amount of material is already accumulated. In the present study mitochondria are shown in chlorophyll-bearing parenchyma, in collenchyma, and in living cells of the vascular bundle of the spinach leaf petiole, as well as in ground parenchyma and all living cells of the vascular bundles of the midrib of lettuce.

From the accounts of existing literature concerning the mitochondria of mesophyll cells, Weier (21) draws the conclusion that mitochondria are highly variable intracellular components. The identification of mitochon-

dria in the literature mentioned is based either upon the study of fixed preparations (Löwschin, 14; von Loui, 13) or upon observations of living material without vital staining (Heitz, 10). Both methods do not permit one to differentiate the cytoplasmic particulates. Mitochondrial methods in plant cells will fix mitochondria, plastids, and spherosomes, while photomicrographs of living unstained cells under the light microscope, using a low voltage lamp and blue filter, will only occasionally produce enough contrast to record the mitochondria. Significantly, however, Heitz (10) mentions the permanent presence in cells of globular structures, presumably spherosomes, of similar size to mitochondria, but distinctly different in appearance and motion.

SUMMARY

More study of cytoplasmic particulates must be made in living material, for this method offers the best hope of understanding their normal metabolic functions. Phase contrast microscopy provides a very valuable aid to the study of the living cell; and, in combination with vital staining, it is possible to observe and often to identify various components of the cytoplasm.

Observations of mitochondria in plant cells by the use of vital Janus green B staining can be confused by the presence in the cells of other granules which may acquire a blue coloring. These granules are either precipitates of A-type vacuoles or spherosomes.

Mitochondria are identified and photographed in epidermal cells, in chlorophyll-bearing parenchyma cells, in collenchyma cells, in the cells of ground parenchyma, and in the living cells of vascular bundles in both lettuce and spinach.

Spherosomes are distinctly identified in the cells of lettuce by phase contrast microscopy, so that confusion with the other particulate is impossible.

Epidermal cells of lettuce were used to study the precipitates of A-type vacuoles, and it was found that by introducing combined staining with Janus green B and Neutral red confusion of the precipitate with mitochondria could be avoided, the mitochondria staining blue, and the precipitate staining red.

No precipitate of the A-type found in some cells of lettuce were found in spinach. Instead, the cells of the petiole exhibited globular precipitates characteristic of B-type vacuoles.

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Winchester, Mass.

EXPLANATION OF PLATES

PLATE I. All photomicrographs were made with phase contrast optics and green filter from living cells of lettuce stained with Janus green B.

FIG. 1. Epidermal cell mounted cutin side up, showing nucleus and distribution of the following cytoplasmic particulates: large oval light green plastids; rod-shaped plastids; very small spherosomes; a few spherical mitochondria. With the exception of the latter, none of the forms was stained with Janus green B. × 956.

FIG. 2. Epidermal cell mounted cutin side down, showing nucleus surrounded by oval green plastids and numerous blue colored mitochondria. Outline of a portion of subepidermal cell is visible in upper part of photograph. × 1069.

FIG. 3. Subepidermal cell, showing oblique transverse walls, nucleus, few green plastids and large number of blue colored mitochondria. × 1190.

FIG. 4. A portion of epidermal cell showing nucleus with a ring of green plastids and numerous spherosomes not stained by Janus green B. × 1105.

FIG. 5. A portion of epidermal cell, exhibiting morphologically similar particulate. Part of the structures were stained with Janus green B (mitochondria) and part were not (colorless plastids). × 1105.

PLATE II. Photomicrographs were made from living cells of lettuce stained either with Janus green B, or the combination of Janus green B with Neutral red. Except fig. 6–7, bright light optics and green filter were employed.

FIG. 6. Epidermal cell stained with Janus green B under phase contrast. Precipitate of A-type vacuole begins to form in different parts of central vacuole (irregular clumps). Nucleus, round plastids and spherosomes are distinct. Mitochondria difficult to recognize. × 731.

FIG. 7. Same cell as in fig. 6, at different focus.

FIG. 8. Epidermal cell stained with combination of Janus green B and Neutral red. Copious, coarse grained, red-colored precipitate and blue colored mitochondria were observed. Nucleus was with a ring of oval plastids. × 979.

FIG. 9. Guard cells. Precipitate dark red, mitochondria blue, plastids green. × 979.

FIG. 10. Subepidermal cell stained with Janus green B. Precipitate brilliant blue with green refraction, finer grained and copious. × 979.

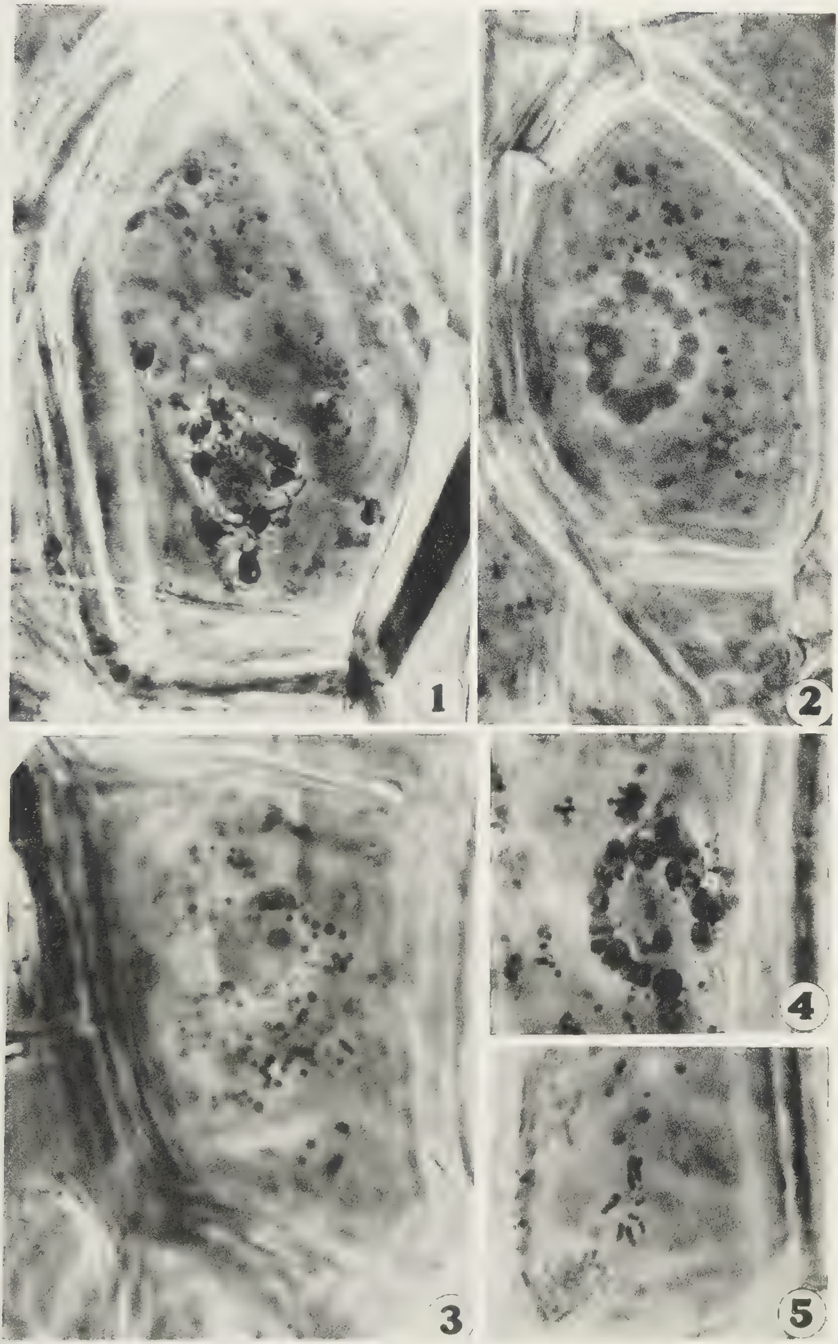
PLATE III. All photomicrographs were taken from living cells of spinach stained either with Janus green B, or the combination of Janus green B with Neutral red. With the exception of FIG. 12, all were taken under bright light optics with green filter.

FIG. 11. Epidermal cell stained with combination of Janus green B and Neutral red, showing green plastids, blue colored mitochondria, and large red colored globular precipitate typical for B-type vacuoles. × 1012.

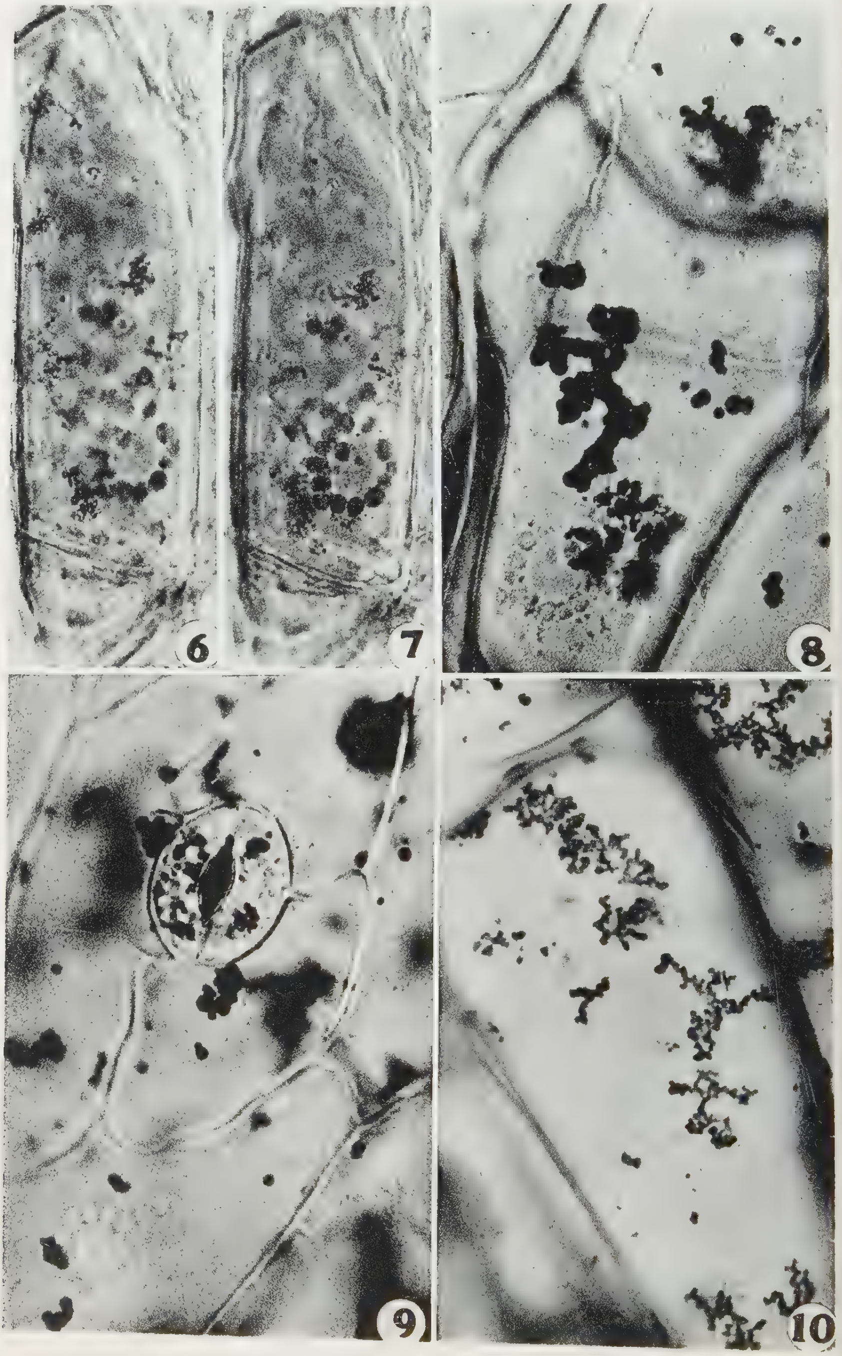
FIG. 12. Same cell as in FIG. 11 photographed under phase contrast optics. The cell shows more depth, but the sharpness of the outline of the precipitate is lost. × 1012.

FIG. 13. A large chlorophyll-bearing parenchyma cell stained with Janus green B shows the distribution of green chloroplasts and blue colored mitochrondria. × 698.

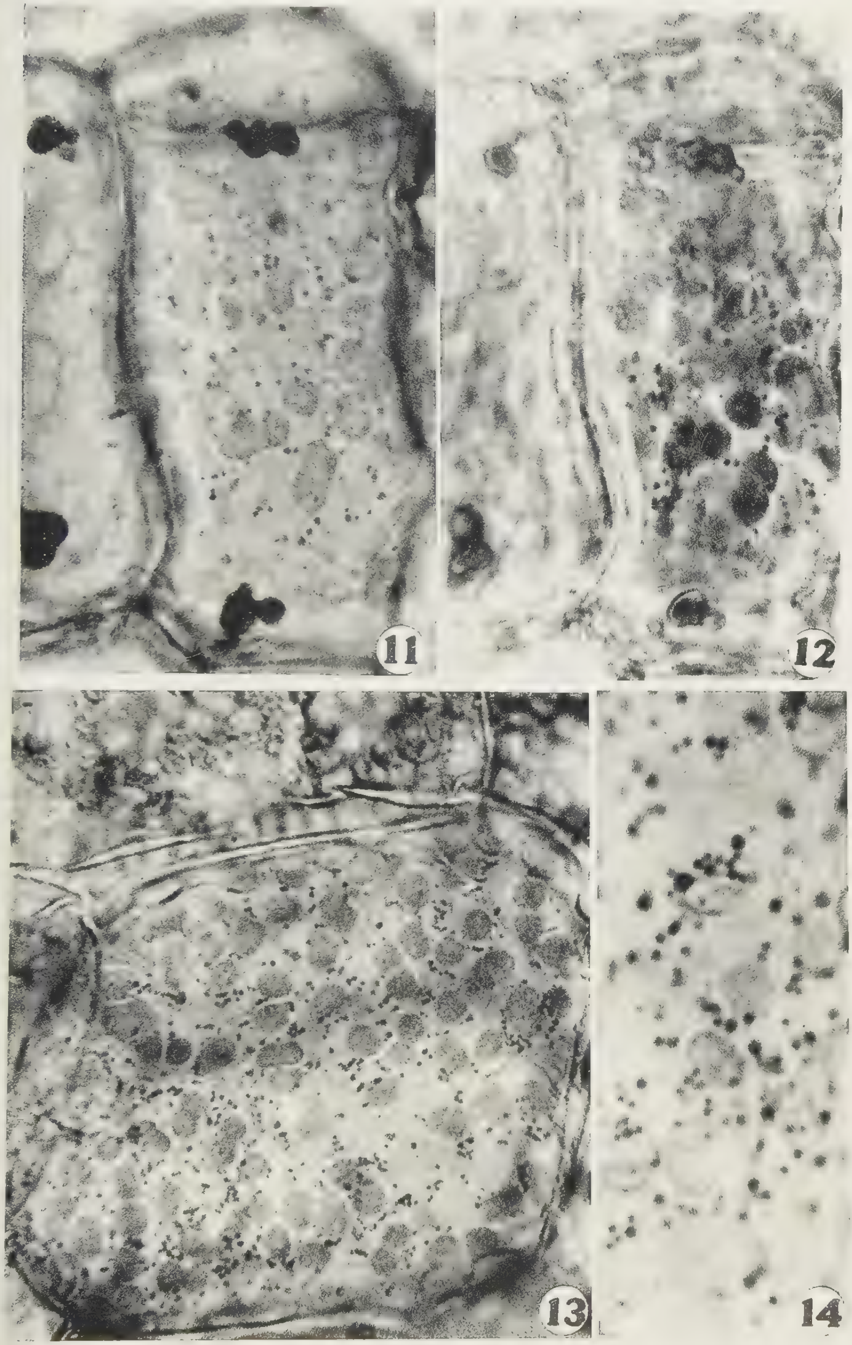
FIG. 14. A portion of chloroplast-containing parenchyma cell under larger magnification, showing chloroplasts and mitochondria. × 1513.



H. Sorokin, Mitochondria and Precipitates



H. Sorokin, Mitochondria and Precipitates



H. Sorokin, Mitochondria and Precipitates

ON THE DIFFERENTIATION OF XYLEM

Ralph H. Wetmore and Sergei Sorokin

With six plates

INTRODUCTION

The Tracheophyta comprise those plants which have vascular tissues. Since xylem and phloem are almost universally associated in the histological composition of vascular tissues, it is probable that their differentiation is activated by substances present in or available to the procambial or cambial regions at essentially the same time. Descriptions now exist of the initiation of primary phloem and xylem in leaf and bud primordia and in adjacent stem tissues for enough angiosperms to suggest that the limiting factors may be different for these two tissues (Jacobs, 1952, 1954). Yet little is known of the identity of these factors in any plant. Evidence (Jacobs, 1952, 1954) supports the belief that an auxin may be of hormonal influence in removing the limitation on xylem differentiation. So far as the authors are aware, nothing is known that establishes the nature of the limiting factors in the differentiation of phloem. Nor has evidence been provided which would designate reasons for the characteristic development of primary xylem and phloem from the procambium rather than from some other tissue. That the severing of mature vascular tissues by deliberate incisions (Jost, 1940, 1942; Sinnott and Bloch, 1944; Jacobs, 1947b, 1952, 1954) can be followed by restored vascular connections across regions where no procambium existed is of arresting importance. Jacobs has recently extended his quantitative studies to show that xylem regeneration following experimental incisions is proportional to the polarized flow of auxin when the latter is in physiological concentrations.

The present investigations are the early results of a study of xylem regeneration in homogenous, parenchymatous callus (a) into which have been grafted the apical portions of shoots of the same species, or (b) instead of the grafted apices, agar containing auxin in varying physiological concentrations takes the place of the scion. Qualitative findings are reported at this time; the work must be extended to give further meaning to these studies.

MATERIAL AND METHODS

The present study has been largely conducted on *in vitro* grown explants of callus of *Syringa vulgaris* L. Preliminary studies of corresponding nature have been made on *Parthenocissus tricuspidata* (Sieb. & Zucc.) Planch. and *Helianthus annuus* L. In *Syringa*, the callus was obtained from the cambial region of the stem by well-established techniques (Gautheret, 1942; Morel,

1948). All the experiments were completed from a single clone of the callus. Callus for the other two species was obtained from sections of the stem (Gautheret, 1942; Morel, 1948).

Syringa callus grows well *in vitro* on the following medium: —

Knop's solution [1]	500 cc.	Cysteine	10 mg.
Double distilled water	350 cc.	Autoclaved coconut milk, 15%	150 cc.
Minor elements [2]	1 cc.	Vitamin-free casein hydrolysate	1 cc.
Ferric citrate	10 mg.	Agar, 1%	10 g.
Sucrose, 5%	50 g.		
Vitamin B mixture [3]	1 cc.		

[1] As modified by Gautheret (1942; Nitsch, 1951).

[2] Stock solution made up according to the formula of Nitsch (1951) with the addition of 25 mg. of Co Cl_2.

[3] The composition of the Vitamin B mixture employed is as follows: — Thiamin hydrochloride 1.0 mg./cc.; Pyrodoxin 1.0 mg./cc.; Nicotinic acid 5.0 mg./cc.; Riboflavin 0.5 mg./cc.; Calcium pantothenate 5.0 mg./cc.; Folic acid 0.1 mg./cc.; Biotin 0.01 mg./cc.; Choline chloride 1.0 mg./cc.; Para-amino-benzoic acid 0.5 mg./cc.; Inositol 10.0 mg./cc.

The same medium fosters the growth of *Parthenocissus* and *Helianthus* cultures, except that certain changes in sucrose (to 6%) and in coconut milk (to 5%), respectively, seem to improve their growth. Callus cultures grow well enough to need transferring at least every three months.

Apices of *Syringa* shoots, usually with three to five pairs of leaf primordia, are prepared for grafting by cutting the base into a wedge, the whole being perhaps 3 to 5 mm. long. A piece of lilac callus is removed from its culture tube into a petri plate, and a V-shaped cut is made into the top of the callus, the sides of the V being kept at about the same slant as those of the wedge-shaped scion to be grafted. Into this cut is put enough agar, or nutrient medium, as called for by the design of the experiment, to fill it.[4] The prepared apex is then pushed into this agar or nutrient agar medium, due regard being given to the orientation of the slanting sides of the apex and of the corresponding walls of the V-shaped cut. When firmly in place, the grafted apex-on-callus is planted in a new tube of medium and left to grow (FIGS. 1–3). In some experiments, no apex is planted in the V-shaped cut; the medium in the cut is characteristically replaced at intervals.

Large pieces of callus with grafted apices are prepared for embedding and sectioning by gashing the surfaces to ensure penetration of reagents into the limited intercellular spaces of the massive callus and to ensure better aspiration in the killing and fixing agent. Craf III is employed for this purpose on a modification containing a higher concentration of acetic acid which seems to give less shrinkage of cells. Materials are embedded in Tissue Mat, sectioned, stained, mounted, and photographed by standard techniques.

[4] So far as the author knows, this technique was first published by Camus (1949). To Dr. G. M. Morel, a co-worker at that time, who knew of and showed him the technique before Camus' publication had been received, the author owes sincere thanks.

GRAFTS OF APEX OF SYRINGA ON SYRINGA CALLUS

Apices of growing lilac branches show visible signs of growth within a week after being grafted into lilac callus. If the medium contains basic mineral salts, minor elements, 2% sucrose, and a low physiological concentration of an auxin (0.1 mg./l. of naphthaleneacetic acid), the callus and the scion grafted into it respond slowly (FIGS. 1, 2). If then transferred to a coconut milk (15%–20%), casein hydrolysate mixture, B-vitamin mixture, sucrose (5%) medium (FIG. 3), growth is rapid in both callus and grafted scion. In fact, within two to three months the scion often develops roots (FIG. 3, right), and the new plant becomes independent of the callus host.

In all, over the past five years, more than 150 grafts of lilac apices on lilac callus have been made, the medium studied and improved, and many resultant growths have been sectioned and studied.[5]

It is interesting, though not unexpected, that the medium proving best for lilac apices by themselves is also that most beneficial for the growth of lilac callus and for scions of lilac grafted into that callus. When two or three apices are grafted into the same callus (FIG. 1), characteristically they all start growing, but, more often than not, one of these outgrows the others; in such cases the effects of the competition become increasingly apparent with time (FIG. 3).

A histological investigation of graft unions shows a well-integrated system. It is difficult to tell from older, established grafts how the union was initiated. A study of young cases suggests no uniformity of behavior. Sometimes cell proliferation occurs on the flanking surfaces of the V-shaped scion; at other times early activity seems largely to be from the sides of the incision in the callus host. The soundness of the technique used by Camus is found in the effect of the agar, whether alone or containing nutrient medium, on the cut cell walls; these are never dry, and seem to be no deterrent to good graft unions. When the grafts have been firmly established and such space as may have existed in the V-shaped cut between host and scion has been filled by cell proliferation, a kind of interdigitation is evident in which outgrowths of either may invade the other. This is especially evident in cases in which lobes from the scion displace neighboring shoot tissue — witness the large bifid-appearing lobe (FIG. 4) below and to the left of the scion, and another at the upper left. The lower lobe is not of proven connection in the figure, but in neighboring sections the connection is very evident.

In order to illustrate a common set of reactions to the influence of the grafted apex, a typical series is illustrated by representative transverse sections of low magnification (FIGS. 5–12), and by enlarged views of critical parts of these sections (FIGS. 13–18). The illustrations have been selected from a study of a 54-day-old graft which had two or three pairs

[5] I want to pay tribute to past students who have had a share in this work, especially Dr. Carl Price and Mrs. Eleanor Gossard Shore.

of leaves left on the apex when dissected for grafting. Originally two apices were grafted into this callus; when killed and fixed the callus was cut in two pieces, each with its scion; both pieces were sectioned and studied, and essentially the story for one is the story for both. In fact, this story would have done for others grown on any medium if the same non-auxin-containing agar was used to fill the cut before the scion was planted. It will be noticed, however, that all proliferation throughout the callus was general and abundant, even as it was extensive and important in the scion itself. It is believed that the description which follows will enable the reader to understand trends and interpretations to be commented on later in the text. TEXT-FIGURE 1 will be helpful. The scion is established in the top 3 mm. of this half of the callus, which is about 12 mm. thick. The cross section pictured below is near the 2 mm. level.

The first section (FIG. 6) is less than 1 mm. below the surface of the callus. The V-shaped cuts are indicated along the nearly horizontal edges of the scion; the nearly vertical edges still are epidermis-covered and therefore uninjured. The vascular cylinder was injured by the cutting preliminary to grafting, but in the proliferated cell masses of the flanks of the scion it has more or less reestablished itself. Certain vascular strands can be seen in the host callus along the cut sides.

Some 500 μ lower (FIGS. 5, 13), the vascular cylinder of the scion has become larger, instead of smaller. An examination of the cylinder at the higher magnification (FIG. 13) suggests an explanation for this change in size. Reactivation of cell division in the pith is supported by the radial rows of cells abutting on the inner sides of the xylem. In fact, this radial extension of the pith has become so pronounced that pressures have disrupted the pith, the newly proliferated scion tissue on the lower flank, and even the callus tissue beyond the limits of the scion. The borders of the scion can be more or less identified by the black-staining remains of the agar placed earlier in the incision. It is noteworthy that the tear seems to have become somewhat filled up with proliferated cells. Subsequent enlarged sections will show this same phenomenon.

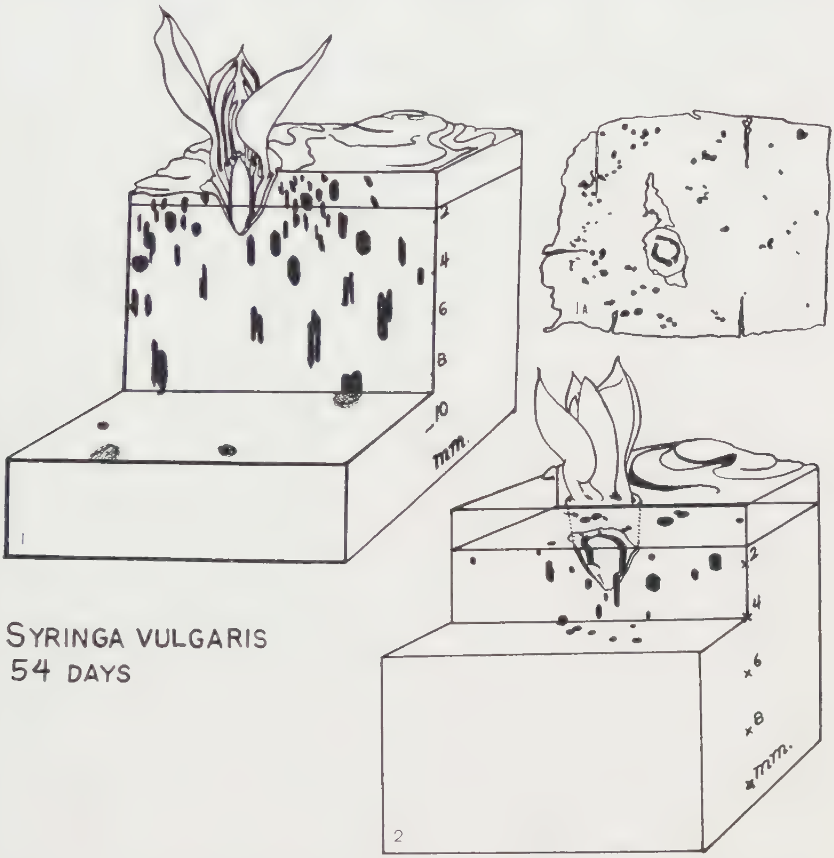
In the next section, almost at the 2 mm. level of FIG. 2 (Figs. 7, 14, 15), the changes of the next 300 μ can be recognized. The arc of vascular tissue on the right of the scion (FIG. 7) is now of two parts. Some of the extensive, radially organized pith has become secondarily thickened and lignified as xylem. For example, the left-hand vascular arc is shown enlarged in FIG. 15. Here one large cell, somewhat oblique, is a vessel segment — marked X — the porous perforation showing clearly at one end. Numerous other elements in this and the neighboring radially arranged rows are circular-bordered in pitting, though not too clear at this magnification.

Mention should be made of the obvious vascular strands in the callus regions near to both flanks of the scion (FIG. 7); these were less numerous in earlier sections (FIGS. 5, 6) but were represented there. (Note TEXT-FIG. 2.)

At approximately 500 μ further (FIGS. 8, 16), more reduction in the vascular tissue of the scion is evident. The split in the pith and adjacent

flanking tissue of the scion stands out, and radial organization of the pith is still obvious, especially on the left (FIG. 16). The indefinite boundary between the tightly knitted scion and the host callus is scarcely to be told on the lower flank in this figure except for the black-stained agar and the seeming contrast in size of cells.

In FIGURE 17, 20 μ lower than FIG. 8 or 70 μ lower than FIG. 16, the reduced vascular tissues in the scion are represented by two circular strands.



TEXT-FIGURE 1. A stereogram of a piece of lilac callus into which was grafted an apex of lilac bearing 2 or 3 pairs of leaf primordia. The incision was filled with 1% agar plus 0.05 mg./l. n.a.a. This drawing is made from a study of a series of transverse sections, when the graft-callus had grown for 54 days. TEXT-FIGURE 1A shows a transverse section at about the 2 mm. mark.

TEXT-FIGURE 2. A second stereogram of another graft showing more clearly the relation of vascular strands in the graft and in the scion below, indicating a near continuity on the right-hand side; in this graft, no auxin was put in the agar placed in the incision. Note the scattered strands in both text-figures.

Radially organized pith still can be detected on the left side. By 100 μ lower, the smaller strand has disappeared, leaving a stretch of radially organized scion parenchyma. In FIGURE 9, about 150 μ lower, the remaining circular vascular strand is much reduced, the only recognizable scion tissue being the small, circular, black agar-delimited area around this strand. The remainder of the section is of callus tissue, with some vascular strands.

About 3.0 mm. below the surface of the callus, or about 0.5 mm. below the bottom of the scion (FIG. 10), a new central bundle can be recognized, situated almost exactly in the position of the round strand (FIGS. 17, 18) which disappeared and was replaced a few sections later (TEXT-FIG. 2) in the callus. This new strand can be followed down for a considerable distance in the callus (FIG. 11) until it is no longer recognizable in the medley of strands present (FIG. 12) a full millimeter below.

Even though vascular strands become increasingly evident at varying distances below the scion, the number diminishes some 6 mm. below the surface or about 3 mm. down from the lower extremity of the scion (TEXT-FIG. 1).

A study of this series and many others of similar kind seems to allow the following inferences: —

1. A scion grafted into callus with no auxin in the agar filling the incision before grafting, whether it is present or not in the sterile medium upon which the apex-callus is grown, tends to (a) produce new vascular tissue in its own proliferated pith tissue, and (b) to stimulate the mitotic activity, which gives rise to nests or short columns of cells in the callus. These columns become organized into vascular strands, often of short vertical extent. Frequently these strands can almost be considered as continuous with those in the scion.

2. The scattered strands of the callus are most numerous below or on the lower flanks of the scion not at greater distances away.

3. The strands, when organized so that mature cells are present, contain lignified, pitted xylem elements, often perforate. No conducting phloem cells have been recognized. Macerations must be carried out, however, before their absence can be made certain.

4. Organization of vascular strands was obviously incomplete when the experimental studies were stopped. Cultures must be maintained longer to learn what further development may produce. Clearly, the characteristic continuous vascular cylinder of the lilac stem is not formed in the callus. Equally clearly, it is hard to picture any relation between organization of vascular strands and conduction.

EFFECTS OF AUXIN-TREATED INCISIONS IN CALLUS

In certain experiments, we used nutrient agar in the incisions instead of plain agar. These nutrient agar media vary in auxin content. In some, we added definite concentrations of synthetic auxins, in others varying amounts of autoclaved coconut milk, with large but unknown concentrations of auxins (Nitsch, 1955). It is evident from these studies that the

concentration of auxin in the incision agar, however added, limits the differentiation of vascular strands around the grafted scion; nor is there any mitotic activity found in the pith of the scion. Rather, vascular strands develop at varying distances from the axis of the scion, these distances having a direct relation to the concentration of auxin present. If higher concentrations are employed in the incision agar, the vascular strands form well out toward the periphery of the callus.

Because auxin produced by the apical meristems, leaves, etc., is significant in the production of vascular tissue in the characteristically parenchymatous callus, it was resolved to leave the scion out completely and study the effects of variations in incision-auxin level alone.

When into an incision was put agar containing 0.25% of naphthalene-acetic acid (n.a.a.) in lanolin and when the whole callus was grown on the best medium now known for *Syringa*, 15% autoclaved coconut milk with its naturally occurring auxin, casein hydrolysate, and 5% sucrose, the callus grew well (See Material and Methods). After four and a half months of active growth, sections showed no vascular tissue anywhere (FIG. 21). This absence of vascular strands is what one characteristically finds in *Syringa* callus on all media studied.

By contrast, when the incision was provided with nutrient medium in agar containing the same content of auxin as indicated above, i.e., 15% autoclaved coconut milk, active arcs of extensive cell proliferation occur in the callus (FIG. 19), which give way to vascular strands.

In another instance of interesting response, the incision was filled with n.a.a. in agar in a concentration of 5 mg./l.; the callus was subsequently grown on the standard medium containing 5% sucrose, vitamin B mixture, 15% autoclaved coconut milk, and 1 cc./l. of casein hydrolysate with the usual mineral-agar background. Great activity was found just within the periphery at the end of thirty-one days (FIG. 20). Material kept longer gives indication of vascular strands being organized in this rectangular, cambial-like zone, as is already suggested in the upper part of FIG. 20.

It would appear that auxin in the incision agar in the range of physiological concentrations without being supplemented by a growing scion can produce those conditions which favor the incidence of vascular tissue in the callus.

DISCUSSION

The apical meristem of the shoot system has at least three well-recognized functions. It adds new cells to the apices. It gives rise to the primordia of new leaves and buds. It plays a part in controlling biochemically the organization of the stem behind it (Ball, 1946, 1948, 1949, 1951, 1952a, 1952b; Wardlaw, 1944, 1947, 1949, 1950, 1952; Wetmore and Wardlaw, 1951; Jacobs, 1952, 1954). Little is known of how these organizational changes are brought about. Evidence has accumulated to suggest that an auxin plays a limiting role in the differentiation, especially so far as it relates to xylem. These auxin effects, so far as known, have recently been

reviewed by Jacobs (1952, 1954). He concludes that in *Coleus* (1952) much more of the auxin has its origin in the young leaves than in the apical meristem itself, leaf number two being the largest contributor. In consequence, once carbohydrate shortage in the upper regions of the plant has been cared for, — this being a second limiting factor as he envisions it — xylem differentiation is initiated, and in the angiosperms at least, in a basipetal direction in keeping with an auxin gradient. Known facts accord with this interpretation. He adds to the earlier work of Simon (1930) and of Kaan-Albest (1934) in finding that there is in this group of plants also a slight and slow acropetal differentiation of xylem as well as that in a basipetal direction. Perhaps a major contribution is in his more recent study (1954). Here he reports that there is in a developing internode a lesser acropetal movement of auxin than basipetal, to a ratio of about 1:3. Broadly, this is in keeping with the contrast between the two aggregates of differentiated xylem, the basipetally developed portion being about three times as much as that developed acropetally.

In the present studies, mitotic activity begins on the cut surfaces of both callus host and the grafted apex within a few days following the operation. In those examples in which the apex is put in auxin-free agar in the incision, the surface activity is followed by a rib meristem-like series of mitoses on either side of the pith, oriented such that the ribs are at right angles to the vascular strands. This development occurs only with the opening of the grafted bud and essentially at the same time as the activity on the surfaces at the graft union. That some of this newly formed, regularly and radially arranged pith, especially on and near the cut flanks of the scion, should become vascular is noteworthy. The implication can only be that something in the experimental procedure has either removed the limitation which kept pith tissue stable or has stimulated it to divide again in orderly fashion. This permits or promotes the next stage from which, as might be expected in lilac, ultimately are produced crowded, circular-bordered, pitted vessel segments with porous perforations in oblique end walls (FIG. 15). The elements are shorter than they are in normal xylem of lilac. One cannot know from these specimens, which were grown as grafts for only fifty-four days, what might have happened had they been grown longer. The experiment must be tried again and for longer periods. For the present, it must be pointed out that no phloem has been detected in the new tissue of this nor like grafts. This seeming absence might be due to failure to recognize it, since careful diagnosis is necessary for identification of phloem either by special stains (Cheadle, Gifford and Esau, 1953) or by maceration. It is proposed to follow the present study with others in which both techniques are invoked.

Soon after the rejuvenation of the pith, and with the closing of the agar-filled incision by the completed graft union, vascular strands begin to appear in that part of the callus flanking the V-shaped cuts, not far from the incisions. The earliest strands arise on the flanks, about three-quarters of the way down the V-cut, and, once initiated as centers of mitotic activity, develop basipetally, quickly acquiring vascular elements of pitted xylem.

These are followed by other strands below the scion almost in continuity with those of the uncut ends. Strands also appear in the callus near the lower edges of the cuticle-covered epidermis of the ends. Later, more strands appear in the callus on the flanks, even farther out and lower down than those reported above (TEXT-FIG. 2). At the time that experiments were stopped — and a 54-day period was the longest any were maintained in culture — the strands were all of short vertical extent. Their ultimate length and possible duration of development are unknown.

It is important to realize that vascular strands in the positions which many of these occupy and with no more continuity than they exhibit could have little significance as water-conducting xylem. One cannot avoid pondering this fact in terms of the pattern of development in normal stems. Is the solution of this problem of the differentiation of xylem in the cylindrical or near-conical axis of the shoot to be found in the fact that continuing strands of elongating procambial cells are preferred paths for auxin movement? If carbohydrates carried by phloem represent a second necessity for xylem differentiation, then one can recognize the significance of the characteristic pattern of phloem differentiation preceding xylem differentiation at any level (Jacobs, 1954). Given carbohydrate, through differentiated phloem elements, and auxin in adequate amounts, it appears that these factors are no longer limiting to xylem differentiation. As it is likely that the favorable concentrations of auxin and carbohydrate are likely to be in the procambium, it is to be expected that xylem formation should be associated with the presence of phloem.

Importance is attached to the fact that vascular strands can experimentally be somewhat generally allocated to regions of the callus. If, instead of a 1% agar as the medium to fill the cut before the scion is inserted, the agar contains a synthetic auxin in known concentration in the physiological range, the vascular strands formed in the callus reflect the auxin concentration. With a concentration of 1 mg. per l., the strands occur only towards the outside of the callus, very few being found near the scion. With 0.01 mg. per l. in the agar placed in the incision, few strands occur near the periphery of the callus — in pieces approximately 1 cm. on a side; they tend to be aggregated on the lower flanks and below the scion. In a broad way, the number and distribution of the strands is dependent upon the concentration of the auxin, at least for either naphthaleneacetic acid or indole-3-acetic acid, the only auxins tried.

When the usual V-shaped cuts are made in the callus, these cuts filled with an auxin-agar mixture, but no apex inserted, results are obtained not dissimilar to those with the apices. A low concentration of auxin (FIG. 21 with 0.25% n.a.a. in lanolin) gives in four and a half months no strands at all. By contrast a medium containing 0.05 mg. per l. of n.a.a. + 15% autoclaved coconut milk, with its high concentration of indole-3-acetic acid, and 5% sucrose (FIG. 19) produces strands scattered in the callus. Few to none of these, however, are found in proximity to the cut, even though the auxin-agar was only replaced once during five and a third months. Again, when 5 mg. per l. of n.a.a. in 1% agar is put into the

incision, a cambial-like zone, very often found in similar cases, is produced toward the outside of the callus, which then resolves itself into strands of xylem, as can be seen (near the upper left, FIG. 20).

In summary, it seems clear that the findings from the early and beautifully planned experiments of Camus (1949) and the more recent and equally significant studies of Sinnott and Bloch (1944) and of Jacobs (1952, 1954) are further supported by the present investigations. It is clear that an auxin placed in or near the top of a callus can make the difference between the formation and the non-formation of vascular strands in which at least xylem differentiates. The role of sugar, as suggested by Jacobs, is not absolutely confirmed, though circumstantial evidence points to its probability. An important problem remains. Why do physiological concentrations of auxin in the agar medium foster the growth of *Syringa* callus *in vitro* but are not effective in the formation of vascular strands in the callus? Yet the same concentrations used in incisions on the upper surface of the agar are accompanied by the appearance of strands. This would seem to suggest a polarity to the callus, as was reported by Gautheret (1940) for carrot tissues, even when isolated from the carrot, and (1941) for endive root tissues. The general handling of callus tissues after a few transfers suggests little polarity, as indicated by a preferential absorption of auxin from the medium. One reasons thus from the fact that growth appears very uniform in pieces planted with little or no regard to their orientation with relation to the original plant. Experiments will be made to investigate the question of polarity of lilac callus in its relation to auxin transfer when initially grown and after varying numbers of passages to new media.

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EXPLANATION OF PLATES

PLATE I

Fig. 1. Habit of *Syringa* apex grafted into *Syringa* callus, 7 weeks old. Graft incision filled with agar containing 2% sucrose and 0.1 mg./l. n.a.a.

Fig. 2. Habit of 19-week, 5-day-old graft of *Syringa* apex into *Syringa* callus. Agar mixture used in graft incision as above.

FIG. 3. Habit of 8.5-months graft of *Syringa* apex into *Syringa* callus. The graft was planted on the medium of FIGS. 1 and 2; at the end of 6 months it appeared about as in FIG. 2. Upon transfer to a medium containing 20% autoclaved coconut milk. casein hydrolysate (1%), it grew to the pictured, internode-bearing plant, rooted (see 2 short roots on right-hand plant) in about 2½ months. Note control apex in the middle, not grafted.

PLATE II

FIG. 4. Longitudinal section of callus-graft union of 5-weeks-old graft. Note black-stained agar around the base of the scion. Two lobes of the scion are shown on the left, the upper cut almost in median plane, the lower not shown in direct connection with the main body of the base of the scion. × 10.

FIG. 5. Transverse section of scion-graft union of a 54-day graft grown on a dextrose, yeast-extract medium with thiamin and cysteine, but no coconut milk nor synthetic auxin; 1% agar used in the incision. Section almost 1.5 mm. below surface of callus-graft union. Note upper and lower flanks of scion are along cut edges of wedge-shaped scion base. The side surfaces of the scion are epidermis-covered. × 11.6. (This section is about 500 μ below FIG. 6, but through an error was mounted out of order. The section labelled FIG. 6 and there described is actually FIG. 5 and that described as FIG. 5 is FIG. 6.)

FIG. 6. Transverse section about 500 μ above FIG. 5. Note increased diameter of vascular cylinder. due to radial multiplication of pith cells (See FIG. 13). × 11.6.

PLATE III

FIG. 7. Transverse section about 300 μ below FIG. 6. Note the arc of the vascular cylinder on the right is disrupted; radial organization of the pith is especially prominent on the left. Note the extra vascular strands on both flanks, above and below. See FIGS. 14, 15 for enlargements from section close to this one. × 11.6.

FIG. 8. Transverse section about 500 μ below FIG. 7. Note the narrowed wedge of the scion; two strands of vascular tissue are left, much decreased in size. Agar is irregularly distributed. As epidermis disappears on right, note appearance of strands in callus. See FIG. 16 for enlargement. × 11.6.

FIG. 9. Transverse section about 150 μ below FIG. 8. The left vascular strand of scion has disappeared, the right. almost so. A small black agar-contained area with remains of right vascular strand is obvious. Some strands are visible in the callus. × 11.6.

PLATE IV

FIG. 10. Transverse section over 0.5 mm. below FIG. 9. Right-hand arc of cylinder has disappeared, but it has been replaced by another almost below it. See TEXT-FIG. 2. Strands in the callus are more pronounced. × 11.6.

FIG. 11. Transverse section about 0.25 mm. below FIG. 10. Note continuing central strand and enlarged strands in the callus. × 11.6.

FIG. 12. Transverse section about 1.0 mm. below FIG. 11. Note increasing prominence of the numerous strands. × 11.6.

PLATE V

FIG. 13. Enlarged view of scion of FIG. 5. Note rows of new cells in pith on both sides; these radial rows have become extensive enough to displace laterally the original vascular tissue, tearing the pith and cortex of the scion and the adjacent callus. × 55.

FIG. 14. Enlarged view of scion of section 40 μ above FIG. 7. Note radial rows on either side of tear in pith. × 55.

FIG. 15. Enlarged view of right-hand part of vascular cylinder of FIG. 14. Note oblique vascular elements in upper part of radial rows; element marked X shows vessel perforation; neighboring elements in side view show crowded, circular bordered pits. × 67.

FIG. 16. Enlarged view of scion from section 50 μ above FIG. 8. Note reduction of arcs of vascular cylinder; radial rows and tears in pith still obvious. × 50.

FIG. 17. Enlarged view of much smaller scion 20 μ below FIG. 8. Note rapid reduction in extent of vascular strands in the 70 μ between FIG. 16 and FIG. 17. Radial organization of pith still present. Note limits of grafted scion as indicated by black agar lines. × 50.

FIG. 18. Enlarged view of transverse section of scion 90 μ below FIG. 17 (or 40 μ above FIG. 9). Note left-hand strand has disappeared leaving the large proliferated parenchymatous mass at base of scion. The right-hand strand is present, circular in outline. × 45.

PLATE VI

FIG. 19. Transverse section of callus cut through remains of V-shaped cut after 5 1/3 months. Cell proliferation has almost filled the incision, only irregular bits remaining. Little of the agar is visible, though it was replaced once. The agar contained 15% coconut milk and 5% sucrose. Note extensive cell proliferation midway out in callus which has given way to vascular tissue. × 4.2.

FIG. 20. Transverse section of 31-day callus after incision in upper surface was filled with agar containing n.a.a. in a concentration of 5 mg. per l. and 5% sucrose. Mixture replaced once. Note absence of vascular strands near incision, and the rectangular cambial-like layer near the periphery. This layer begins to show strands in the upper left. × 10.4.

FIG. 21. Transverse section of a 4½-months' callus grown on a favorable medium with an incision in the top filled with 0.25% n.a.a. in lanolin which was replaced once. Note the absence of vascular strands over the whole callus; auxin diffuses much more slowly from lanolin than from auxin.

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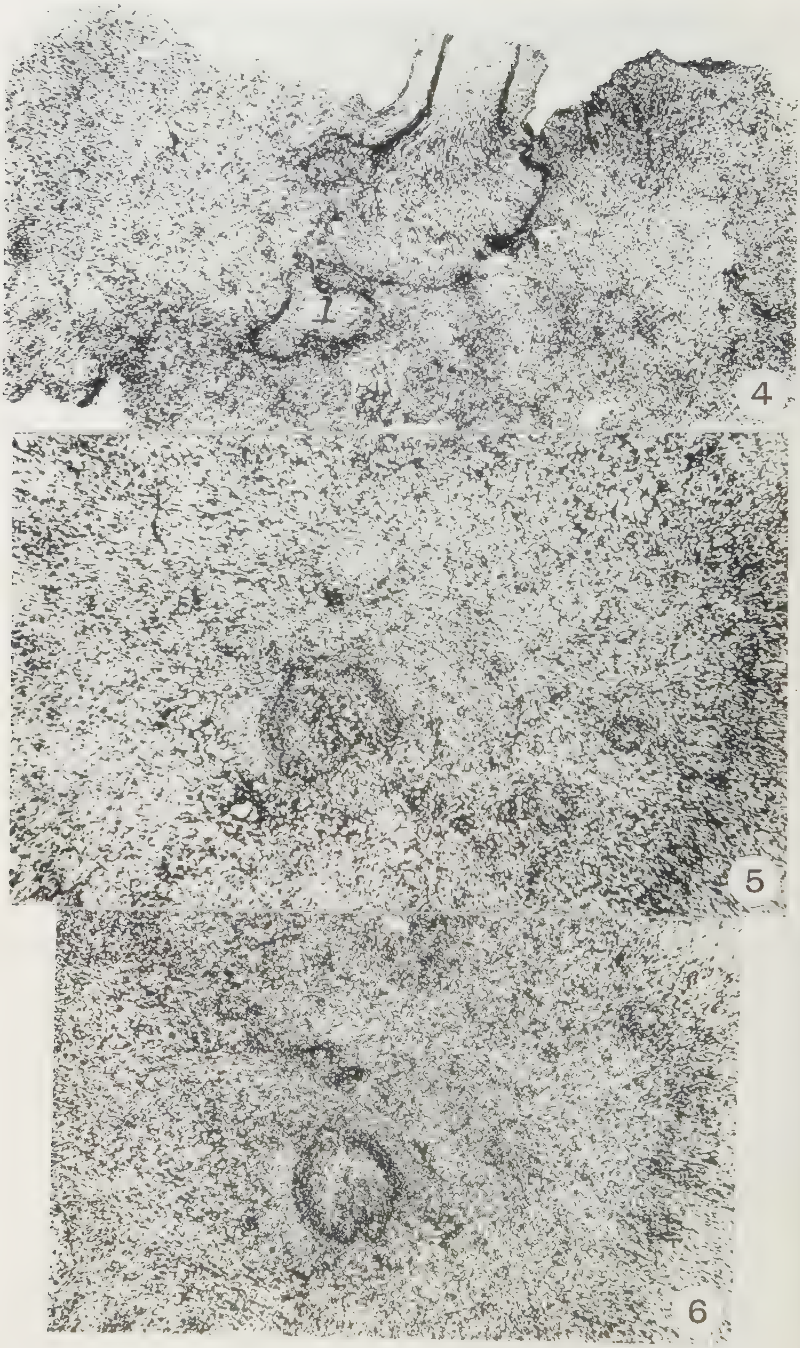

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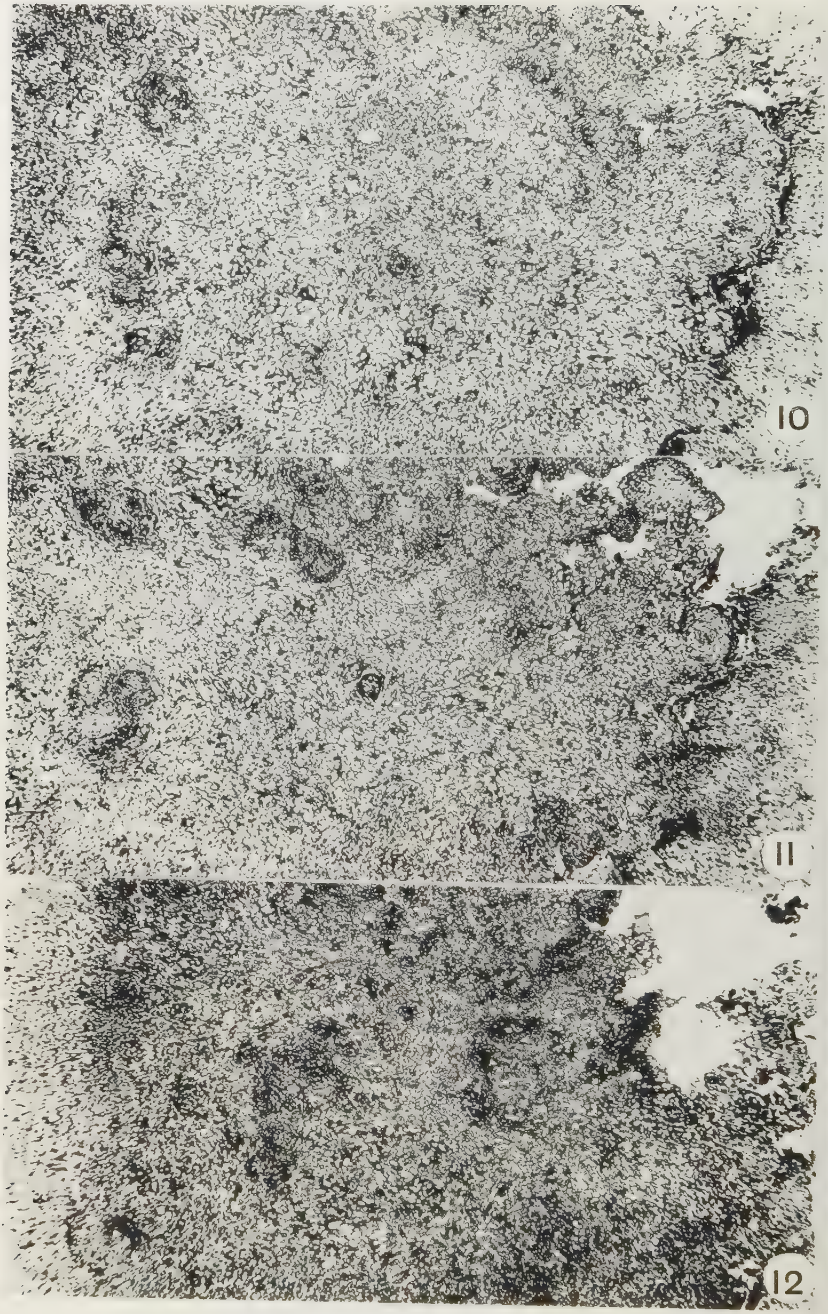

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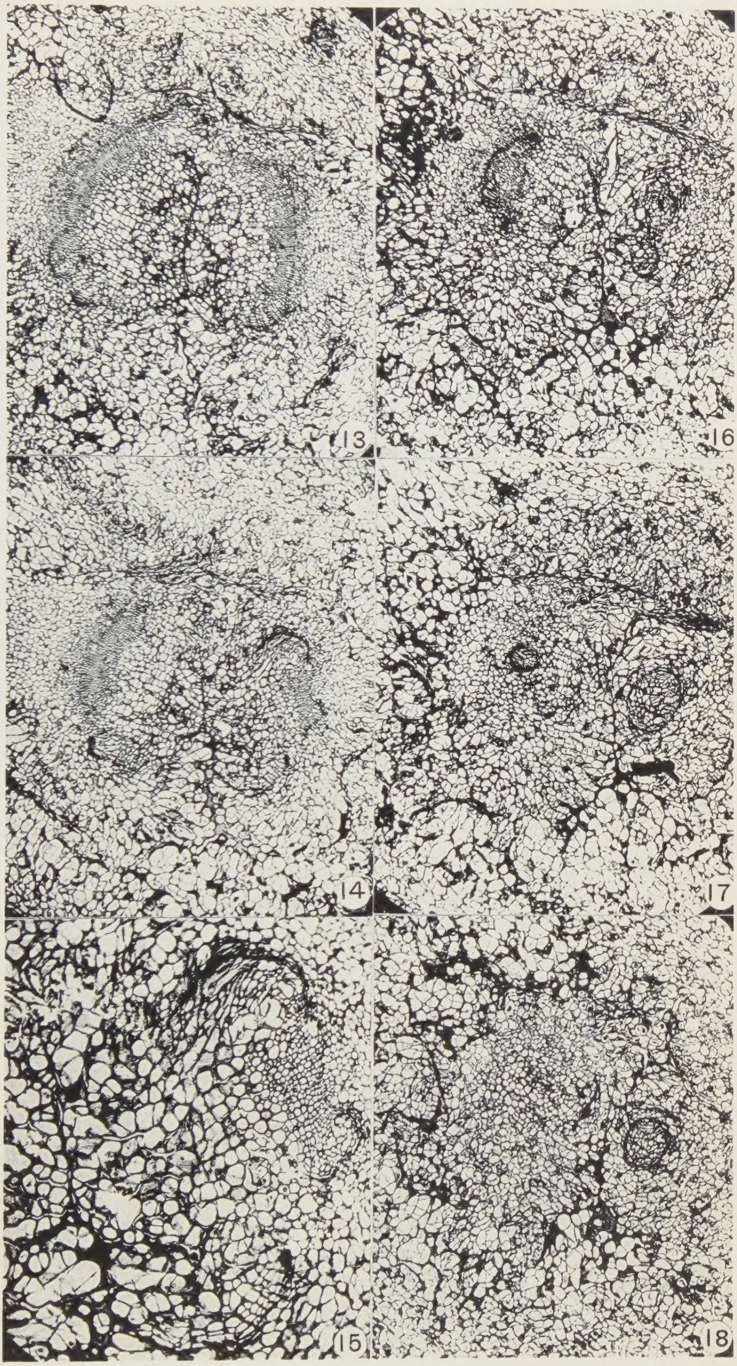

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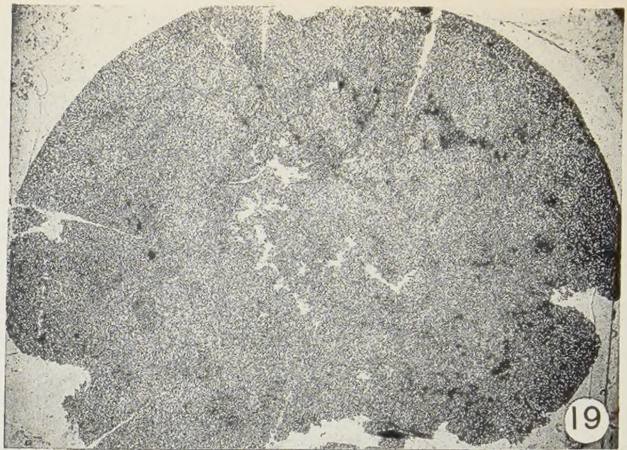

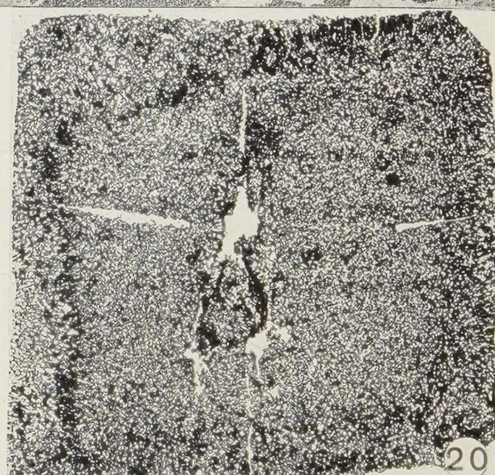

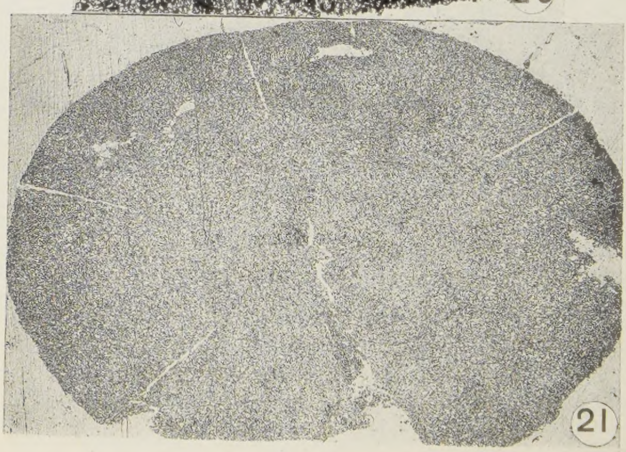

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